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(54) Title: AUTOIMMUNE CONDITIONS AND NADPH OXIDASE DEFECTS

(57) Abstract: The invention relates to methods and materials involved in diagnosing and treating autoimmune conditions. In particular, the invention relates to methods and materials involved in diagnosing arthritis conditions that are accompanied by an NADPH oxidase deficiency, methods and materials involved in treating, preventing, or delaying the onset of arthritis conditions that are accompanied by an NADPH oxidase deficiency, and methods and materials involved in identifying agonists and antagonists of NADPH oxidase activity.

AUTOIMMUNE CONDITIONS
AND NADPH OXIDASE DEFECTS

RELATED APPLICATIONS

5   This application claims the benefit of U.S. Provisional Application Serial No.
60/380,904, filed May 13, 2002 and U.S. Provisional Application Serial No. 60/429,609,
filed November 27, 2002.

BACKGROUND

10   *1. Technical Field*

The invention relates to methods and materials involved in diagnosing and
treating autoimmune conditions. In particular, the invention relates to methods and
materials involved in diagnosing arthritis conditions that are accompanied by an NADPH
oxidase deficiency, methods and materials involved in treating, preventing, or delaying

15   the onset of arthritis conditions that are accompanied by an NADPH oxidase deficiency,
and methods and materials involved in identifying agonists and antagonists of NADPH
oxidase activity.

2. Background Information

20   Autoimmune conditions are conditions where a mammal's immune system starts
reacting against its own tissues. Such conditions include, without limitation, arthritis
(e.g., rheumatoid arthritis (RA)), multiple sclerosis, lupus, autoimmune uveitis, type I
diabetes, bronchial asthma, septic arthritis induced with staphylococci or streptococci,
and cardiovascular disease involving vasculitis.

25   RA is a chronic inflammatory disease that can be found in about 1-2 % of the
population. RA primarily affects peripheral joints where inflammatory synovitis leads to
cartilage destruction, bone erosion, and ultimately to joint deformity and loss of joint
function. RA is a complex disease in that both environmental factors as well as multiple
chromosomal regions are involved in susceptibility to RA. Inducers of arthritis in animal

30   models include adjuvants, collagen (e.g., collagen type II) (collagen induced arthritis
(CIA)), hexadecane (hexadecane induced arthritis (HIA)), oil (e.g., Freund's incomplete

adjuvant), squalene (squalene induced arthritis (SIA), and pristane (pristane induced
arthritis (PIA)). Chromosomal regions known to be associated with development of RA
include the major histocompatibility complex region. In addition, different genomic
regions are known to control different phases of the disease such as onset, severity during
5   the acute onset phase, and the severity of the destruction in the chronic relapsing phase.

SUMMARY

The invention provides methods and materials related to diagnosing and treating
autoimmune conditions such as arthritis (e.g., RA), multiple sclerosis, lupus, autoimmune
10   uveitis, type I diabetes, bronchial asthma, septic arthritis induced with staphylococci or
streptococci, and cardiovascular disease involving vasculitis. For example, the invention
provides methods and materials involved in diagnosing autoimmune conditions that are
accompanied by an NADPH oxidase deficiency, methods and materials involved in
treating, preventing, and/or delaying the onset of autoimmune conditions that are
15   accompanied by an NADPH oxidase deficiency, and methods and materials involved in
identifying agonists and antagonists of NADPH oxidase activity. Autoimmune
conditions that are accompanied by an NADPH oxidase deficiency include, without
limitation, arthritis (e.g., RA), multiple sclerosis, lupus, autoimmune uveitis, type I
diabetes, bronchial asthma, septic arthritis induced with staphylococci or streptococci,
20   and cardiovascular disease involving vasculitis conditions that coexist with or are caused
by a deficiency in NADPH oxidase activity. For the purpose of this invention, the term
"arthritis accompanied by NADPH oxidase deficiency" (abbreviated "AANOD") refers to
any arthritis condition that coexists with or is caused by a deficiency in NADPH oxidase
activity. Such a deficiency can be the complete lack of NADPH oxidase activity or a
25   partial reduction in NADPH oxidase activity. For example, a mammal can have AANOD
when that mammal has arthritis as well as cells that exhibit NADPH oxidase activity to an
extent less than that normally exhibited by healthy mammals of the same species.
Likewise, a mammal can have multiple sclerosis accompanied by an NADPH oxidase
deficiency when that mammal has multiple sclerosis as well as cells that exhibit NADPH
30   oxidase activity to an extent less than that normally exhibited by healthy mammals of the
same species.

It is noted that the methods and materials for diagnosing and treating AANOD described herein can be used to diagnose and treat other autoimmune conditions that are accompanied by NADPH oxidase deficiency. For example, the methods and materials described herein can be used to diagnose and treat multiple sclerosis conditions that are

5   accompanied by NADPH oxidase deficiency. In this regard, experimental allergic (autoimmune) encephalomyelitis (EAE) is a useful model for multiple sclerosis.

Diagnosing patients having an autoimmune condition accompanied by an NADPH oxidase deficiency (e.g., AANOD) can help clinicians determine appropriate treatments for those patients. For example, a clinician who diagnoses a patient as having AANOD

10   can treat that patient with medication that improves both the patient's arthritis and NADPH oxidase deficiency. In some cases, a single medication can be used to improve a patient's level of NADPH oxidase activity such that the patient's arthritis symptoms are reduced or relieved. Thus, treating a patient having AANOD by modulating NADPH oxidase activities can improve that patient's health and quality of life by, for example,

15   reducing the symptoms or severity of symptoms associated with arthritis, or delaying the onset of arthritis.

In addition, identifying agonists and antagonists of NADPH oxidase activity can help both clinicians and patients. For example, the methods and materials described herein can be used to identify agents that increase NADPH oxidase activity such that

20   patients with an autoimmune condition accompanied by an NADPH oxidase deficiency (e.g., AANOD) can be treated successfully.

The invention is based on the discovery that arthritis can be associated with or caused by a reduced level of NADPH oxidase activity. For example, development of severe arthritis symptoms in an arthritis animal model can be, at least partially, dependent

25   upon the presence of low NADPH oxidase activity. The invention also is based on the discovery that the reduced level of NADPH oxidase activity responsible for arthritis susceptibility can be caused by sequence variations (e.g., mutated phosphorylation sites) in a polypeptide component (e.g., P47PHOX polypeptide) of a mammal's NADPH oxidase enzyme. In addition, the invention is based on the discovery that mammals prone

30   to develop arthritis can be protected by providing those mammals with normal levels of NADPH oxidase activity. For example, an animal model prone to develop severe arthritis

symptoms can be rescued by providing that animal with a fully functional NADPH oxidase pathway.

In general, the invention features a method for assessing a mammal's susceptibility to develop an autoimmune condition (e.g., arthritis or multiple sclerosis),

5 the method includes: (a) providing, from a mammal, a blood or synovial fluid sample containing a cell; (b) determining the level of NADPH oxidase activity of the cell after contacting the cell with an NADPH oxidase activator; (c) determining whether or not the level is less than a control level of NADPH oxidase activity, wherein the control level is the average amount of NADPH oxidase activity of control cells from a population of

10 mammals without the autoimmune condition (e.g., non-arthritic mammals), and wherein the mammals without the autoimmune condition are from the same species as the mammal; and (d) identifying the mammal as being susceptible to develop the autoimmune condition when the level is less than the control level. The NADPH oxidase activator can be norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol

15 myristate acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, hexadecene, heptadecane, octadecane, galectin 1, or galectin 3. The level of NADPH oxidase activity can be determined by measuring superoxide or reactive oxygen species. The population can contain at least 10 mammals. Step (c) can include determining whether or not the level is

20 between 5 and 75 percent less than the control level, wherein step (d) includes identifying the mammal as being susceptible to develop the autoimmune condition when the level is between 5 and 75 percent less than the control level. Step (c) can include determining whether or not the level is between 25 and 55 percent less than the control level, wherein step (d) includes identifying the mammal as being susceptible to develop the autoimmune

25 condition when the level is between 25 and 55 percent less than the control level.

In another embodiment, the invention features a method for assessing a mammal's susceptibility to develop an autoimmune condition (e.g., arthritis or multiple sclerosis), the method containing: (a) providing, from a mammal, a blood or synovial fluid sample; (b) determining the level of a blood or synovial fluid component that reflects NADPH

30 oxidase activity; (c) determining whether or not the level is less than a control level, wherein the control level is the average amount of the component in control samples from

4

a population of mammals without the autoimmune condition (e.g., non-arthritic
mammals), and wherein the mammals without the autoimmune condition are from the
same species as the mammal; and (d) identifying the mammal as being susceptible to
develop the autoimmune condition when the level is less than the control level. The
5   component that reflects NADPH oxidase activity can be malonic dialdehyde.

Another embodiment of the invention features a method for assessing a mammal's
susceptibility to develop an autoimmune condition (e.g., arthritis or multiple sclerosis),
the method containing determining whether or not a mammal contains a genetic variant of
the gene encoding a polypeptide that functions in the NADPH oxidase pathway, where
10   the presence of the genetic variant indicates that the mammal is susceptible to develop the
autoimmune condition. The genetic variant can encode a mutant polypeptide. The mutant
polypeptide can be a GP91PHOX polypeptide, P22PHOX polypeptide, P40PHOX
polypeptide, P47PHOX polypeptide, or P67PHOX polypeptide. For example, the mutant
polypeptide can be a P47PHOX polypeptide. The mammal can be a human, and the
15   mutant P47PHOX polypeptide can contain the sequence set forth in SEQ ID NO:6 with at
least two amino acid substitutions. The mutant P47PHOX polypeptide can contain an
amino acid sequence having an amino acid residue other than valine at the position that
aligns with position 106 of SEQ ID NO:4 or an amino acid residue other than threonine at
the position that aligns with position 153 of SEQ ID NO:4. The genetic variant can
20   contain a mutation in a regulatory sequence of the gene.

Another embodiment of the invention features a method for diagnosing an
autoimmune condition accompanied by NADPH oxidase deficiency (e.g., AANOD or
multiple sclerosis accompanied by NADPH oxidase deficiency) in a mammal having an
autoimmune condition, the method containing: (a) providing, from the mammal, a sample
25   containing a cell; (b) determining the level of NADPH oxidase activity of the cell after
contacting the cell with an NADPH oxidase activator; (c) determining whether or not the
level is less than a control level of NADPH oxidase activity, wherein the control level is
the average amount of NADPH oxidase activity of control cells from a population of
mammals without the autoimmune condition (e.g., non-arthritic mammals), and wherein
30   the mammals without the autoimmune condition are from the same species as the
mammal; and (d) identifying the mammal as having the autoimmune condition associated

by NADPH oxidase deficiency when the level is less than the control level. The NADPH oxidase activator can be norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, hexadecene,

5   heptadecane, octadecane, galectin 1, or galectin 3. The level of NADPH oxidase activity can be determined by measuring superoxide or reactive oxygen species. The population can contain at least ten mammals without the autoimmune condition. Step (c) can include determining whether or not the level is between 5 and 75 percent less than the control level, wherein step (d) includes identifying the mammal as being susceptible to develop

10   the autoimmune condition associated by NADPH oxidase deficiency when the level is between 5 and 75 percent less than the control level. Step (c) can include determining whether or not the level is between 25 and 55 percent less than the control level, wherein step (d) includes identifying the mammal as being susceptible to develop the autoimmune condition associated by NADPH oxidase deficiency when the level is between 25 and 55

15   percent less than the control level.

       Another embodiment of the invention features a method for diagnosing an autoimmune condition associated by NADPH oxidase deficiency (e.g., AANOD or multiple sclerosis accompanied by NADPH oxidase deficiency) in a mammal having an autoimmune condition, the method containing: (a) providing, from the mammal, a blood

20   or synovial fluid sample; (b) determining the level of a blood or synovial fluid component that reflects NADPH oxidase activity; (c) determining whether or not the level is less than a control level, wherein the control level is the average amount of the component in control samples from a population of mammals without the autoimmune condition (e.g., non-arthritic mammals), and wherein the mammals without the autoimmune condition are

25   from the same species as the mammal; and (d) identifying the mammal as having the autoimmune condition associated by NADPH oxidase deficiency when the level is less than the control level.

       Another embodiment of the invention features a method for diagnosing an autoimmune condition accompanied by NADPH oxidase deficiency (e.g., AANOD or

30   multiple sclerosis accompanied by NADPH oxidase deficiency) in a mammal having an autoimmune condition, the method includes: (a) providing a blood or synovial fluid

sample from the mammal; (b) determining the level of a blood or synovial fluid component that reflects NADPH oxidase activity; (c) determining whether or not the level is less than a control level of NADPH oxidase activity, wherein the control level is the average amount of NADPH oxidase activity of control cells from a population of

5   mammals without the autoimmune condition, and wherein the mammals without the autoimmune condition are from the same species as the mammal; and (d) identifying the mammal as having the autoimmune condition accompanied by NADPH oxidase deficiency when the level is less than the control level. The component that reflects NADPH oxidase activity can be malonic dialdehyde.

10   Another embodiment of the invention features a method for diagnosing an autoimmune condition accompanied by NADPH oxidase deficiency (e.g., AANOD or multiple sclerosis accompanied by NADPH oxidase deficiency) in a mammal having an autoimmune condition, the method including determining whether or not the mammal contains a genetic variant of the gene encoding a polypeptide that functions in the

15   NADPH oxidase pathway, wherein the presence of the genetic variant indicates that the mammal has the autoimmune condition accompanied by NADPH oxidase deficiency. The genetic variant can encode a mutant polypeptide. The mutant polypeptide can be a GP91PHOX polypeptide, P22PHOX polypeptide, P40PHOX polypeptide, P47PHOX polypeptide, or P67PHOX polypeptide. For example, the mutant polypeptide can be a

20   P47PHOX polypeptide. The mammal can be a human, and the mutant P47PHOX polypeptide can contain the sequence set forth in SEQ ID NO:6 with at least two amino acid substitutions. The mutant P47PHOX polypeptide can contain an amino acid sequence having an amino acid residue other than valine at the position that aligns with position 106 of SEQ ID NO:4 or an amino acid residue other than threonine at the

25   position that aligns with position 153 of SEQ ID NO:4. The genetic variant can contain a mutation in a regulatory sequence of the gene.

In another aspect, the invention features a method for treating a mammal having an autoimmune condition accompanied by NADPH oxidase deficiency (e.g., AANOD or multiple sclerosis accompanied by NADPH oxidase deficiency), the method including

30   administering, to the animal, an agent that enhances NADPH oxidase activity. The agent can be norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate

acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane, galectin 1, or galectin 3. Alternatively, the agent can be a more polar derivative of the afore-mentioned compounds. For example, the agent can be an alkene derivative of the afore-mentioned

5   compounds (e.g., undecene, hexadecene), or an acid derivative of the afore-mentioned compounds. In one embodiment, hexadecene is used. In another embodiment, undecane is used. The agent may be administered intra-dermally, intra-peritoneally, or intra-nasally.

In another embodiment, the invention features the use of an agent in the

10   manufacture of a medicament to treat an autoimmune condition accompanied by NADPH oxidase deficiency (e.g., AANOD or multiple sclerosis accompanied by NADPH oxidase deficiency), wherein the agent enhances NADPH oxidase activity in a mammal. The agent can be norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane,

15   undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane, galectin 1, or galectin 3. Alternatively, the agent can be a more polar derivative of the afore-mentioned compounds. For example, the agent can be an alkene derivative of the afore-mentioned compounds (e.g., undecene, hexadecene), or an acid derivative of the afore-mentioned compounds. In one embodiment, hexadecene is used. In another

20   embodiment, undecane is used.

Another embodiment of the invention features a method of formulating a medicament for the treatment of an autoimmune condition, the method including: (a) contacting a sample comprising cells or a cellular fraction having NADPH activity with a test agent, (b) determining the level of NADPH oxidase activity in the sample, (c)

25   determining whether or not the level is greater than a control level of NADPH oxidase activity, wherein the control level is the amount of NADPH oxidase activity in a control sample lacking the test agent, (d) identifying the test agent as an agent useful for treatment of the autoimmune condition when the level of NADPH oxidase activity is greater than the control level, and (e) formulating a medicament from the agent for the

30   treatment of the autoimmune condition. The autoimmune condition can be arthritis or multiple sclerosis.

Another aspect of the invention features a method for identifying an agent that activates NADPH oxidase activity in a cell, wherein the cell is from a non-human animal susceptible to arthritis induction, the method containing determining whether or not the level of NADPH oxidase activity increases in the cell after the cell is treated with a test

5   agent, wherein an increase in the level indicates that the test agent activates NADPH oxidase activity. The cell can be a cell from a DA rat. The cell can be a lymphocyte. The non-human animal can be susceptible to pristane induced arthritis, or collagen induced arthritis, or adjuvant induced arthritis, or oil induced arthritis, or hexadecane induced arthritis, or squalene induced arthritis, or avridine induced arthritis. The level of

10   NADPH oxidase activity can be determined by measuring superoxide or reactive oxygen species, e.g., as with a cytochrome C assay or WST-1 assay or a flow cytometer (e.g., FASC) based assay using, e.g., dihydrorodamine 123 (DHR-123).

In another embodiment, the invention features a method for identifying an agent useful in the treatment of an autoimmune condition, the method including: (a) contacting

15   a sample comprising cells or a cellular fraction having NADPH activity with a test agent, (b) determining the level of NADPH oxidase activity in the sample, (c) determining whether or not the level is greater than a control level of NADPH oxidase activity, wherein the control level is the amount of NADPH oxidase activity in a control sample lacking the test agent, and (d) identifying the test agent as an agent useful for treatment of

20   the autoimmune condition when the level of NADPH oxidase activity is greater than the control level. The autoimmune condition can be arthritis or multiple sclerosis.

In another embodiment, the invention features a method for identifying an agent useful in the treatment of an autoimmune condition, the method including: (a) contacting a sample comprising cells or a cellular fraction having NADPH activity with a test agent,

25   (b) contacting the sample with an NADPH oxidase activator, (c) determining the level of NADPH oxidase activity in the sample, (d) determining whether or not said level is greater than a control level of NADPH oxidase activity, wherein the control level is the amount of NADPH oxidase activity in a control sample treated with the activator in the absence of the test agent, and (e) identifying the test agent as an agent useful for treatment

30   of the autoimmune condition when the level of NADPH oxidase activity is greater than the control level. The autoimmune condition can be arthritis or multiple sclerosis.

In another embodiment, the invention features a method for identifying an agent that enhances NADPH oxidase activity in a cell, wherein the cell is from a non-human animal susceptible to arthritis induction, the method including: (a) contacting the cell with an NADPH oxidase activator and a test agent to form a test cell; (b) determining the level

5   of NADPH oxidase activity in the test cell; (c) determining whether or not the level is greater than a control level of NADPH oxidase activity, wherein the control level is the amount of NADPH oxidase activity in a control cell treated with the activator in the absence of the test agent; and (d) identifying the agent as enhancing NADPH oxidase activity when the level is greater than the control level. The cell can be a cell from a DA

10   rat. The cell can be a lymphocyte. The non-human animal can be susceptible to pristane induced arthritis or collagen induced arthritis, or adjuvant induced arthritis, or oil induced arthritis, or hexadecane induced arthritis, or squalene induced arthritis, or avridine induced arthritis. The NADPH oxidase activator can be norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine,

15   fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, hexadecene , heptadecane, octadecane, galectin 1, or galectin 3. The level of NADPH oxidase activity can be determined by measuring superoxide or reactive oxygen species, e.g., by a cytochrome C or WST-1 assay or a flow cytometer (e.g., FASC) based assay using, e.g., dihydrorodamine 123 (DHR-123).

20   In another embodiment, the invention features a method for identifying an agent that inhibits NADPH oxidase activity in a cell, wherein the cell is from a non-human animal susceptible to arthritis induction, the method containing: (a) contacting the cell with an NADPH oxidase activator and a test agent to form a test cell; (b) determining the level of NADPH oxidase activity in the test cell; (c) determining whether or not the level

25   is less than a control level of NADPH oxidase activity, wherein the control level is the amount of NADPH oxidase activity in a control cell treated with the activator in the absence of the test agent; and (d) identifying the agent as inhibiting NADPH oxidase activity when the level is less than the control level. The cell can be a cell from a DA rat. The cell can be a lymphocyte. The non-human animal can be susceptible to pristane

30   induced arthritis, or collagen induced arthritis, or oil induced arthritis, or hexadecane induced arthritis, squalene induced arthritis, or avridine induced arthritis. The NADPH

oxidase activator can be norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, hexadecene, heptadecane, octadecane, galectin 1, or galectin 3. The level of NADPH oxidase activity

5   can be determined by measuring superoxide or reactive oxygen species.

In another embodiment, the invention features a method for identifying an agent that modulates T-cell activation (e.g., reduces or enhances T-cell activation), the method including: (a) determining whether or not a test agent increases NADPH oxidase activity; and (b) classifying the test agent as an agent that reduces T-cell activation when the test

10   agent increases NADPH oxidase activity.

Another aspect of the invention features a rat congenic to a second rat, wherein at least one locus differs genetically between the rat and the second rat, wherein the second rat is susceptible to arthritis induction, wherein the rat contains T-cells from the second rat, and wherein the rat has arthritis. The rat can be a DA.E3c12-/- rat. The second rat

15   can be a DA rat. The at least one locus can contain nucleic acid that encodes a P47PHOX polypeptide. In some embodiments, no more than one locus can differ genetically between the rat and the second rat. In those cases, the locus can contain nucleic acid that encodes a P47PHOX polypeptide.

Another aspect of the invention features a nonhuman mammal having a deficient

20   NADPH oxidase pathway, where the nonhuman mammal exhibits symptoms of an autoimmune disease. The autoimmune disease may be arthritis, multiple sclerosis, lupus, autoimmune uveitis, type I diabetes, bronchial asthma, septic arthritis induced with staphylococci or streptococci, or cardiovascular disease involving vasculitis. For example, the nonhuman animal may exhibit symptoms of arthritis. The arthritis may be

25   adjuvant induced arthritis, collagen induced arthritis, pristane induced arthritis, hexadecane induced arthritis, avridine induced arthritis, or squalene induced arthritis, or oil induced arthritis. The deficient NADPH oxidase pathway may be indicated by a reduced NADPH oxidase activity. The reduced NADPH oxidase activity may be a result of a mutant polypeptide where the mutant polypeptide functions in the NADPH oxidase

30   pathway. For example, the mutant polypeptide may be a GP91PHOX polypeptide, P22PHOX polypeptide, P40PHOX polypeptide, P47PHOX polypeptide, or P67PHOX

polypeptide. For example, the mutant polypeptide can be a P47PHOX polypeptide. The mutant P47PHOX polypeptide can contain the sequence set forth in SEQ ID NO:6 with at least two amino acid substitutions. The mutant P47PHOX polypeptide can contain an amino acid sequence having an amino acid residue other than valine at the position that

5   aligns with position 106 of SEQ ID NO:4 or an amino acid residue other than threonine at the position that aligns with position 153 of SEQ ID NO:4. In other embodiments, the reduced NADPH oxidase activity may be a result of a deletion of the gene or locus encoding Ncf1 (*p47phox*). The deletion may be heterozygous or homozygous in the nonhuman mammal. The nonhuman mammal may be a mouse.

10   In another embodiment, the invention features a method of screening an agent to determine if the agent delays the onset of arthritis. The method includes: (a) providing a nonhuman mammal having a deficient NADPH oxidase pathway; (b) administering to the nonhuman mammal the agent; (c) inducing arthritis in the nonhuman mammal; and (d) determining if the agent delays the onset of arthritis in the nonhuman mammal. The

15   deficient NADPH oxidase pathway may be as described previously. Determining if the agent delays the onset of arthritis may include steps such as: (a) determining a day of onset of arthritis value for the nonhuman mammal; and (b) comparing the day of onset of arthritis value for the nonhuman mammal with a control day of onset of arthritis value. The control day of onset of arthritis value may be determined by determining a day of

20   onset of arthritis value for a control nonhuman mammal to which the agent has not been administered. The day of onset of arthritis in the nonhuman mammal may be considered delayed if it is later than the control day of onset value. The induced arthritis may be adjuvant induced arthritis, collagen induced arthritis, pristane induced arthritis, hexadecane induced arthritis, avridine induced arthritis, squalene induced arthritis, and/or

25   oil induced arthritis.

Another method of the invention includes screening an agent to determine if the agent treats arthritis. The method includes (a) providing a nonhuman mammal having a deficient NADPH oxidase pathway, where the nonhuman mammal exhibits symptoms of an arthritis (e.g., adjuvant induced arthritis, collagen induced arthritis, pristane induced

30   arthritis, hexadecane induced arthritis, avridine induced arthritis, squalene induced arthritis, or oil induced arthritis); (b) administering to the nonhuman mammal the agent;

and (c) determining if the agent treats arthritis in the nonhuman mammal. Such a determining step may involve (a) calculating an arthritis score in the nonhuman mammal; and (b) comparing the arthritis score with a control arthritis score. The control arthritis score may be determined by calculating an arthritis score for a control nonhuman

5   mammal to which the agent has not been administered. The agent may be determined to treat arthritis if the arthritis score in the nonhuman animal is less than the control arthritis score.

In another embodiment, a method of screening an agent to determine if the agent prevents arthritis is provided. The method includes: (a) providing a nonhuman mammal

10   having a deficient NADPH oxidase pathway; (b) administering to the nonhuman mammal the agent; (c) administering a compound known to induce arthritis to the nonhuman mammal; and (d) determining if the agent prevents arthritis induced by the compound in the nonhuman mammal. Determining if the agent prevents arthritis may include evaluating said nonhuman mammal for symptoms of arthritis. Such an evaluation

15   may occur for a period of time, e.g., for up to 20 days, up to 30 days, up to 50 days, or up to 70 days. Determining if the agent prevents arthritis may include comparing any symptoms of arthritis and their day of onset with the symptoms and day of onset of a control nonhuman mammal to which said agent has not been administered. The compound known to induce arthritis may be an adjuvant, collagen, pristane, hexadecane,

20   avridine, squalene, and/or oil. Collagen may be type II collagen; the oil may be incomplete Freund's adjuvant; and the adjuvant may be mycobaterial-derived.

In another embodiment, a method for identifying an agent that inhibits NADPH oxidase activity in a lymphocyte, wherein the lymphocyte is from a DA rat. The method includes: (a) contacting the lymphocyte with PMA and a test agent to form a test cell; (b)

25   determining the level of NADPH oxidase activity in the test cell by measuring superoxide; (c) determining whether or not the level is less than a control level of NADPH oxidase activity, wherein the control level is the amount of NADPH oxidase activity in a control cell treated with the PMA in the absence of the test agent; and (d) identifying the agent as inhibiting NADPH oxidase activity when the level is less than the

30   control level.

In another embodiment, a non-DA rat comprising heterologous T-cells from a non-human animal susceptible to arthritis induction, wherein the non-DA rat has arthritis. The rat can be an E3 rat. The non-human animal can be a DA rat.

Unless otherwise defined, all technical and scientific terms used herein have the

5   same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In

10   case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

15   **DESCRIPTION OF DRAWINGS**

Figure 1 is a graph comparing the mean clinical arthritis scores determined at day 10, 14, 19, 24, and 28 after pristane injection of DA rats, DA.E3chr12+/- rats, and DA.E3chr12-/- rats.

Figure 2 is a physical map of the *Pia4* region constructed using PAC clones and

20   EST clones.

Figure 3 is an alignment of the E3 and DA *p47phox* cDNA sequences amplified from the RNA of E3 and DA rats.

Figure 4 is a set of bar graphs demonstrating that plasma levels of cartilage oligomeric matrix protein (COMP; Figure 4A) and $\alpha 1$-acid glycoprotein (AGP; Figure

25   4B) were significantly lower in DA.E3chr12 congenic rats than in DA littermate control rats (p<0.005).

Figure 5 is a set of absorbance curves illustrating production of reactive oxygen species in a DA rat (Figure 5A), an E3 rat (Figure 5B), a DA.E3c12+/- rat (Figure 5C), and a DA.E3c12-/- rat (Figure 5D).

30   Figure 6 is a graph illustrating increased severity of arthritis in rats treated with an NADPH inhibitor, diphenyleneiodonium chloride (DPI).

Figure 7 is a bar graph illustrating that rats treated with DPI had greater accumulated arthritis scores than control rats.

Figure 8 is a bar graph illustrating that transfer of Conconavalin A (ConA) activated T-cells from a DA rat gave rise to severe arthritis in DA littermate control rats

5   (DA), in DA.E3chr12 +/- rats (HET), and in DA.E3chr12 -/- rats (E3).

Figure 9 is a set of graphs showing the activation of the oxidative burst from rat E3 peritoneal cells (Figure 9A) and HL60 cells (Figure 9B) after treatment with alkanes *in vitro*. The oxidative burst is measured with the WST-1 assay.

Figure 10 is a set of graphs demonstrating the arthritis inducing effects of alkanes

10   12-31 days after administration. Figure 10A demonstrates the Mean score; Figure 10B demonstrates the Additive score; and Figure 10C demonstrates the Maximum score according to the extended scoring system described herein after injection of the alkanes intradermally at the base of the tail.

Figure 11 is a set of graphs demonstrating the severity of PIA in DA rats treated

15   with undecane or undecanol at varying time points. Figure 11A demonstrates the Mean score; Figure 11B demonstrates the Additive score; and Figure 11C demonstrates the Maximum score.

Figure 12 is a set of graphs demonstrating the severity of hexadecane induced arthritis after treatment with hexadecene on day –5 or day +5. Figure 12A demonstrates

20   the Mean score; Figure 12B demonstrates the Additive score; and Figure 12C demonstrates the Maximum score.

Figure 13 is a bar graph demonstrating the severity of arthritis in rats treated with radioactively- labeled oils.

Figure 14 is a bar graph illustrating the tissue distribution of 14C-labeled oils at

25   10, 20, and 30 days post-injection. The activity was measured in a β-counter for one minute. Note that LN = lymph nodes.

Figure 15 is a bar graph illustrating the distribution of [1-14C]-hexadecane to the lymph nodes as measured in a β-counter (Beckman) for one minute.

Figure 16 is a set of bar graphs demonstrating the effect of phytol on the

30   development of EAE in DA rats treated with phytol on day –10, -5 or day +5. Figure 16A

demonstrates the Additive score; Figure 16B demonstrates the Maximum score. Olive oil was used as a control.

Figure 17 is a set of graphs demonstrating the effect of phytol treatment on development of PIA with different routes of phytol administration. Figure 17A

5   demonstrates the Mean score; Figure 17B demonstrates the additive score; and Figure 17C demonstrates the Maximum score.

Figure 18 is a set of graphs illustrating the onset of CIA in Ncf1 (*p47phox*) deficient mice, both homozygous and heterozygous, as compared to B10.Q mice. Figure 18A demonstrates the Mean score; Figure 18B demonstrates the Maximum score; and

10   Figure 18C demonstrates the Additive score.

Figure 19 is a bar graph showing the mean day of onset of CIA in Ncf1 deficient mice, both homozygous and heterozygous, as compared to B10.Q mice.

Figure 20 is a bar graph showing the serum COMP levels in Ncf1 deficient mice, both homozygous and heterozygous, as compared to B10.Q mice following induction of

15   CIA by injection of rat CII.

Figure 21 is a bar graph showing the levels of anti collagen antibodies in Ncf1 deficient mice, both homozygous and heterozygous, as compared to B10.Q mice following induction of CIA by injection of rat CII.

Figure 22 is a graph demonstrating the effect of phytol treatment of CIA using

20   different routes of administration.

Figure 23 is a set of graphs demonstrating the effect of treatment of active PIA using different active agents and routes of administration. 200 μL of either phytol (Figure 23A) or undecane (Figure 23B) were administered at day 21 and day 26.

25   **DETAILED DESCRIPTION**

The invention provides methods and materials related to diagnosing and treating autoimmune conditions (e.g., arthritis). Specifically, the invention provides methods and materials involved in diagnosing mammals susceptible to arthritis and mammals having AANOD. In addition, the invention provides methods and materials involved in treating

30   mammals susceptible to arthritis and mammals having AANOD. Further, the invention

provides methods and materials involved in identifying agonists and antagonists of NADPH oxidase activity.

1. Diagnosing Mammals Susceptible to Arthritis

5   The invention provides methods for assessing a mammal's susceptibility to developing arthritis. The mammal can be a human, monkey, goat, horse, cow, pig, dog, cat, mouse, or rat. Briefly, a mammal's susceptibility to developing arthritis can be determined by examining the level of NADPH oxidase activity present within the mammal's cells. This level of NADPH oxidase activity then can be compared with a

10   control level, and the mammal can be classified as being susceptible to developing arthritis if the level of NADPH oxidase in the mammal's cells is lower than the control level as further described below.

The level of NADPH oxidase activity in a mammal's cells can be determined using any known method. For example, NADPH oxidase activity can be assessed by

15   measuring the amount of reactive oxygen species generated. As used herein, the term "reactive oxygen species" includes, without limitation, partially reduced species of oxygen such as superoxide ion (O_2^-*), hydrogen peroxide (H_2O_2), hydroxyl radical (OH*) and hydroxide ion. The amount of reactive oxygen species generated can be measured using standard methods such as those that involve measuring cytochrome C reduction,

20   lucigenin luminescence, luminol-luminescence, and DCFDA fluorescence. Alternatively, the level of NADPH oxidase activity in a mammal's cells can be determined by measuring the level of components known to reflect NADPH oxidase activity. Such components include, without limitation, circulating malonic dialdehyde.

After determining the level of NADPH oxidase activity present within the

25   mammal's cells, this level of NADPH oxidase activity can be compared with a control level of NADPH oxidase activity for that particular species. The control level of NADPH oxidase activity for a particular species is the average level of NADPH oxidase activity measured in cells from a population of healthy members from that particular species. In the case of humans, the control level of NADPH oxidase activity can be the average level

30   of NADPH oxidase activity in cells from 5, 10, 20, 30, 40, 50, or more healthy humans. If the level of NADPH oxidase activity in a mammal's cells is lower than the control

level, then the mammal can be classified as being susceptible to arthritis. For example, a mammal having an NADPH oxidase activity that is no more than about 85 (e.g., no more than 75, 65, 55, 45, 35, 25, 15, 5, or less) percent of the control level can be classified as being susceptible to arthritis.

5   Alternatively, a mammal (e.g., human) having impaired NADPH oxidase activity can be classified as being susceptible to arthritis. For example, a human having impaired NADPH oxidase activity such that a sample of about 1×10^6 granulocytes from that human exhibits less than 0.3 to 0.4 absorbance units (550 nm) of cytochrome C reduction after about 7 minutes of treatment with 0.01 μM fMLP can be classified as being

10   susceptible to arthritis. In one embodiment, a human having cells with no more than about 85 (e.g., no more than 75, 65, 55, 45, 35, 25, 15, 5, or less) percent of this level of activity is classified as being susceptible to arthritis. The cells can be treated with activators other than fMLP such as agents that affect cellular signaling by modulating phosphorylation (e.g., PMA), agents that destabilize cell membranes (e.g., pristane,

15   squalene, phytol, and hexadecane), and agents that bind cell surface receptors (e.g., galectin1 and galectin 3).

Any type of sample can be used to determine the level of NADPH oxidase activity in a mammal's cells. For example, the sample can be blood, synovial fluid, or lymph fluid containing cells (e.g., PBMCs) having NADPH oxidase activity. Cells having

20   NADPH oxidase activity include, without limitation, macrophages, neutrophils, granulocytes, polymorphonuclear leukocytes, and mononuclear cells. Standard methods can be used to obtain such samples from the mammal. For example, a blood sample can be obtained by venous puncture. The sample can be subjected to any necessary standard preparatory procedures before assessing NADPH oxidase activity. For example, a blood

25   sample containing cells can be subjected to centrifugation and/or washing steps to isolate cells from which the level of NADPH oxidase activity can be measured.

In another embodiment, a mammal's susceptibility to developing arthritis can be determined by examining at least a portion of the amino acid sequence of a polypeptide within the mammal's NADPH oxidase pathway. Such polypeptides include, without

30   limitation, GP91PHOX polypeptides, P22PHOX polypeptides, P40PHOX polypeptides, P47PHOX polypeptides, and P67PHOX polypeptides. For example, the amino acid

sequence of the mammal's P47PHOX polypeptide can be determined. Any method can be used to determine the amino acid sequence of a polypeptide. For example, standard amino acid sequencing techniques can be used to determine the amino acid sequence of a purified P47PHOX polypeptide preparation. Alternatively, the nucleic acid encoding the

5 polypeptide can be sequenced using standard nucleic acid sequencing techniques. Once the nucleic acid sequence is determined, the amino acid sequence of the encoded polypeptide can be deduced.

After determining at least a portion (e.g., about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 percent) of the amino acid sequence of a polypeptide within the mammal's

10 NADPH oxidase pathway, that amino acid sequence can be compared to the amino acid sequence of a comparable reference polypeptide that functions in the NADPH oxidase pathway such that at least about 70 percent (e.g., at least about 75, 80, 85, 90, 95, or 99 percent) of maximal NADPH oxidase activity is observed. For example, the amino acid sequence of a mammal's P47PHOX polypeptide can be compared to the amino acid

15 sequence of a P47PHOX polypeptide that allows a cell to exhibit at least about 70 percent of maximal NADPH oxidase activity. Likewise, the amino acid sequence of a mammal's GP91PHOX polypeptide can be compared to the amino acid sequence of a GP91PHOX polypeptide that allows a cell to exhibit at least about 70 percent of maximal NADPH oxidase activity.

20 The term "maximal NADPH oxidase activity" as used herein refers to the average maximum level of NADPH oxidase activity measured in cells from healthy members of a particular species. For example, in rats, the maximal NADPH oxidase activity when measuring superoxide release from about 5 x 10^6 peritoneal neutrophils using cytochrome C reduction can be 0.15-0.25 absorbance (550 nm) units at about 1000 seconds following

25 stimulation with PMA. The cells can be treated with activators other than PMA such as fMLP, agents that destabilize cell membranes (e.g., pristane, squalene, phytol, and hexadecane), and agents that bind cell surface receptors (e.g., galectin1 and galectin 3). Since cells from E3 rats exhibit at least about 70 percent of this activity, the amino acid sequences of polypeptides that function in the NADPH oxidase pathway of the E3 rat can

30 be used as reference polypeptides. For example, a rat's amino acid sequence of a P47PHOX polypeptide can be compared to the amino acid sequence of the P47PHOX

polypeptide of E3 rats, which is set forth in SEQ ID NO:4. In humans, the maximal
NADPH oxidase activity when measuring superoxide release from about 1×10^6
granulocytes using cytochrome C reduction can be 0.3 to 0.4 absorbance (550 nm) units
about 7 minutes following stimulation with 0.01 μM of fMLP. Thus, polypeptides that

5   function in the NADPH oxidase pathway of human cells that exhibit at least 70 percent of
that activity can be used as reference polypeptides. When assessing a human's P47PHOX
polypeptide, the human P47PHOX polypeptide having the amino acid sequence set forth
in SEQ ID NO:6 can be used as a reference polypeptide.

If the mammal being tested contains a mutant polypeptide when compared to a

10   comparable reference polypeptide, then that mammal can be classified as being
susceptible to developing arthritis. The mutant polypeptide can be a polypeptide that
contains amino acid additions, subtractions, substitutions, or combinations thereof when
compared to the sequence of a comparable reference polypeptide. For example, a mutant
polypeptide can be a polypeptide having any number of amino acid differences (e.g., 1, 2,

15   3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 125, 150, 175,
200, or more amino acid additions, subtractions, or substitutions) when compared to a
comparable reference polypeptide. Thus, in one embodiment, a human can be classified
as being susceptible to developing arthritis if that human contains a P47PHOX
polypeptide having an amino acid sequence with one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9,

20   10, 11, 12, 13, 14, 15, or more) amino acid substitutions when compared to the amino
acid sequence set forth in SEQ ID NO:6. Alternatively, a human can be classified as
being susceptible to developing arthritis if that human contains a P47PHOX polypeptide
having an amino acid sequence with an amino acid residue other than valine at the
position that aligns with position 106 of SEQ ID NO:4.

25   In addition, a mutant polypeptide can be a polypeptide lacking one or more
phosphorylation sites. Typically, phosphorylation sites are serine, threonine, or tyrosine
residues. Thus, a polypeptide lacking one or more serine, threonine, or tyrosine residues
when compared to a comparable reference polypeptide can be a mutant polypeptide. In
one embodiment, a human can be classified as being susceptibility to develop arthritis if

30   that human contains a P47PHOX polypeptide having an amino acid sequence lacking one
or more (e.g., more than 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the serine, threonine, or tyrosine

residues when compared to the amino acid sequence set forth in SEQ ID NO:6.
Alternatively, a human can be classified as being susceptible to developing arthritis if that
human contains a P47PHOX polypeptide having an amino acid sequence with an amino
acid residue other than threonine at the position that aligns with position 153 of SEQ ID
5   NO:4.

In addition to using the presence of a mutant polypeptide to determine whether or
not a particular mammal is susceptible to arthritis, the regulatory sequences (e.g.,
promoters, enhancers, and silencers) that control the expression of a polypeptide that
functions in the NADPH oxidase pathway can be examined. For example, the promoter
10   sequences that control P47PHOX polypeptide expression can be compared to those
promoter sequences that drive normal P47PHOX polypeptide expression in healthy
humans. In this case, a human having a mutated regulatory promoter sequence can be
classified as being susceptible to developing arthritis.

15   *2. Diagnosing Mammals Having AANOD*

The invention provides methods for determining whether a mammal has a
particular type of arthritis. Specifically, a mammal can be diagnosed as having AANOD
if that mammal has (1) clinical symptoms of arthritis and (2) cells having a level of
NADPH oxidase activity that is either lower than a control value or impaired. In
20   addition, a mammal can be diagnosed as having AANOD if that mammal has (1) clinical
symptoms of arthritis and (2) a polypeptide that functions in the NADPH oxidase
pathway and that contains a mutation when compared to a comparable reference
polypeptide as described herein.

Clinical symptoms of arthritis include, without limitation, inflammation of
25   tendons, ligaments, joints, or bones. Symptoms of arthritis also include pain, swelling,
and stiffness in the limbs that can lead to weakness, loss of mobility, and deformity in the
mammal. Examples of arthritis include, without limitation, bacterial arthritis,
osteoarthritis, rheumatoid arthritis (RA), collagen-induced arthritis (CIA), hexadecane-
induced arthritis (HIA), pristane-induced arthritis (PIA), avridine-induced arthritis,
30   adjuvant induced arthritis, squalene-induced arthritis (SIA), and oil-induced arthritis
(OIA).

The level of NADPH oxidase activity within a mammal's cells can be assessed as
described herein. Likewise, the methods and materials described herein can be used to
determine whether or not a mammal contains a mutant polypeptide that functions in the
NADPH oxidase pathway.

5

3. Treating arthritis

The invention provides methods and materials for treating arthritis (e.g., AANOD)
in a mammal. Methods for treating arthritis such as AANOD include administering an
agent that increases the level of NADPH oxidase activity in the mammal. For example,

10 an agent that increases a cell's production of reactive oxygen species can be administered
to a mammal with arthritis. Such agents include, without limitation, norfloxacin,
phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate,
lysophosphatidylcholine, fMLP, octane, decane, undecane, dodecane, tridecane,
tetradecane, hexadecane, heptadecane, octadecane; agents that destabilize cell membranes

15 (e.g., pristane, squalene, phytol, and hexadecane), and agents that bind cell surface
receptors (e.g., galectin1 and galectin 3). Alternatively, the agent can be a more polar
derivative of the afore-mentioned compounds. For example, the agent can be an alkene
derivative of the afore-mentioned compounds (e.g., undecene, hexadecene), or an acid
derivative of the afore-mentioned compounds. In one embodiment, hexadecene is used.

20 In another embodiment, undecane is used.

Agents that increase NADPH oxidase activity can be administered in any standard
form using any standard method. For example, agents that increase NADPH oxidase
activity can be in the form of tablets or capsules (e.g., time-release capsules) that are
taken orally. Alternatively, the agents can be in a liquid form and can be taken orally or

25 by injection. The agents also can be in the form of suppositories. Further, agents that
increase NADPH oxidase activity can be in the form of creams, gels, and foams that can
be applied to the skin. In addition, the agents can in the form of an inhalant that is
applied nasally. The agent may be administered intra-dermally, intra-peritoneally, or
intra-nasally.

30 Agents that increase NADPH oxidase activity can be administered at any dose that
is sufficient to increase NADPH oxidase activity in cells that have low activity. Such

doses can be taken over a period of years to prevent and/or delay the progression arthritis or to reverse the progression of arthritis. Doses can be selected based on the effectiveness and toxicity of the particular agent using standard pharmacology techniques.

5   *4. Identifying Agent That Modify NADPH Oxidase Activity*

The invention provides methods and materials for identifying agents that modulate NADPH oxidase activity. Agents that modulate NADPH oxidase activity can increase or decrease NADPH oxidase activity. Examples of agents that increase NADPH oxidase activity include, without limitation, norfloxacin, phosphatidic acid, diacylglycerol,

10   arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine, fMLP, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane; agents that destabilize cell membranes (e.g., pristane, squalene, phytol, and hexadecane), and agents that bind cell surface receptors (e.g., galectin1 and galectin 3). Examples of agents that decrease NADPH oxidase activity include, without limitation,

15   diphenylene iodonium, phenols, apocynin, quinone, haem ligands, piroxicam, B220 (2,3-dimethyl-6(2-dimethylaminoethyl)-6H-indolo-(2,3-b)quinoxaline), lidocaine, gliotoxin, hydrocortisone, OPC-6535 (6-[2-(3,4-diethoxyphenyl)thiazol-4-yl]-pyridine-2-carboxylic acid), and cromolyn.

To identify agents that increase or decrease NADPH oxidase activity, a test agent

20   can be mixed with a sample containing cells or cellular fractions having NADPH oxidase activity. Such cells can be from humans (e.g., healthy humans or arthritis patients) or non-human animals (e.g., healthy non-human animals or non-human animals susceptible to arthritis such as those susceptible to arthritis induction.) An animal is susceptible to arthritis induction if that animal develops an arthritis condition in response to treatment

25   with an inducing agent (e.g., collagen or pristane). Such animals include those susceptible to CIA, PIA, HIA, SIA, and OIA. The non-human animal can be any type of animal including, without limitation, monkeys (e.g., chimpanzees), horses, goats, cows, pigs, and rodents (e.g., mice and rats).

An example of a non-human animal that is susceptible to arthritis is a rat that is

30   susceptible to PIA such as the DA rat. A rat can be identified as a member of the DA strain (or any strain of interest) using standard methods. For example, standard nucleic

acid sequencing techniques can be used to compare genomic or mitochondrial nucleic acid sequences including, without limitation, (1) microsatellite sequences, (2) nucleic acid sequences encoding major histocompatibility complexes, or (3) nucleic acid sequences encoding 18S ribosomal RNA. Comparing nucleic acid sequence from two animals can

5   be accomplished using genetic analysis tools such as restriction fragment length polymorphism-(RFLP) based methods and random amplified polymorphic DNA-(RAPD) based methods. Two animals can be concluded to be of the same strain if the nucleic acid sequences of both animals have similar characteristics when analyzed by RFLP or RAPD.

10   After being treated with the test agent, the level of NADPH oxidase activity can be determined. The sample can be any type of sample containing a cell or a cellular fraction having NADPH oxidase activity. The sample can be blood, lymph, or synovial fluid. The cell can be a lymphocyte, granulocyte (e.g., neutrophil) or macrophage.

        The NADPH oxidase activity determined in the presence of a test agent can be

15   compared with the NADPH oxidase activity determined in the absence of the test agent. Agents that increase NADPH oxidase activity are those that lead to an increase in NADPH oxidase activity by any amount (e.g., a 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 200, or more percent increase) when compared with the level of NADPH oxidase activity observed in the absence of the test agent. Agents that decrease NADPH oxidase activity

20   are those that lead to a decrease in NADPH oxidase activity by any amount (e.g., a 5, 10, 20, 30, 40, 50, 75, or more percent decrease) when compared with the level of NADPH oxidase activity observed in the absence of the test agent.

        In another embodiment, agents that increase or decrease NADPH oxidase activity can be identified in an assay mixture that includes NADPH oxidase, an activator, and a

25   test agent. In these embodiments, NADPH oxidase activity, determined in the presence of an activator and a test agent, can be compared with the NADPH oxidase activity determined in the presence of the activator without the test agent. As described herein, agents that increase NADPH oxidase activity are those that, when present in the NADPH oxidase assay mixture, lead to an increase in NADPH oxidase activity by any amount

30   (e.g., a 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 200, or more percent increase) when compared with the level of NADPH oxidase activity observed in the absence of the test

agent, while agents that decrease NADPH oxidase activity are those that, when present in
the NADPH oxidase assay mixture, lead to a decrease in NADPH oxidase activity by any
amount (e.g., a 5, 10, 20, 30, 40, 50, 75, or more percent decrease) when compared with
the level of NADPH oxidase activity observed in the absence of the test agent.

5

5. Non-DA Rats Containing Heterologous T-cells

The invention provides methods and materials for generating an animal (e.g., a
rodent such as a rat or mouse) that has heterologous T-cells in combination with a new
form of inducible arthritis (i.e., T-cell induced arthritis; TIA). TIA can be generated in an
10   animal by introducing into the recipient animal, T-cells from a donor animal that has
arthritis. The donor and recipient animals can be from different strains or can be
members of the same inbred group of animals that differ only with respect to (1) the QTL
that contributes to arthritic susceptibility such as the *Pia4* QTL or (2) the sequence of the
p47phox gene. T-cells can be isolated from an animal using any standard procedures
15   including spleen homogenization. T-cell transfer can be performed using any
conventional procedure. An animal with TIA can be used as described herein to identify
agents that modify NADPH oxidase activity.

6. Nonhuman mammals having deficient NADPH oxidase pathways

20   Another aspect of the invention features methods and materials for providing a
nonhuman mammal having a deficient NADPH oxidase pathway, where the nonhuman
mammal exhibits symptoms of an autoimmune disease. The mammal may be a monkey,
goat, horse, cow, pig, dog, cat, mouse, or rat. The autoimmune disease may be arthritis,
multiple sclerosis, lupus, autoimmune uveitis, type I diabetes, bronchial asthma, septic
25   arthritis induced with staphylococci or streptococci, or cardiovascular disease involving
vasculitis. For example, the nonhuman mammal may exhibit symptoms of arthritis. The
arthritis may be induced by standard techniques known in the art and may be, e.g.,
adjuvant induced arthritis, CIA, PIA, HIA, avridine induced arthritis, SIA, or OIA.

The deficient NADPH oxidase pathway in the nonhuman mammal may be
30   indicated by a reduced NADPH oxidase activity. The reduced NADPH oxidase activity
may be a result of a mutant polypeptide where the mutant polypeptide functions in the
NADPH oxidase pathway. For example, the mutant polypeptide may be a GP91PHOX

polypeptide, P22PHOX polypeptide, P40PHOX polypeptide, P47PHOX polypeptide, or P67PHOX polypeptide. For example, the mutant polypeptide can be a P47PHOX polypeptide. The mutant P47PHOX polypeptide can contain the sequence set forth in SEQ ID NO:6 with at least two amino acid substitutions. The mutant P47PHOX

5   polypeptide can contain an amino acid sequence having an amino acid residue other than valine at the position that aligns with position 106 of SEQ ID NO:4 or an amino acid residue other than threonine at the position that aligns with position 153 of SEQ ID NO:4. In other embodiments, the reduced NADPH oxidase activity may be a result of a deletion of the gene or locus encoding Ncf1 (*p47phox*). The deletion may be heterozygous or

10 homozygous in the nonhuman mammal. In one embodiment, the nonhuman mammal may be a mouse. For example, the mouse may have the gene for Ncf1 (*p47phox*) knocked out using standard techniques in the art, or the mouse may express one of the mutant P47PHOX polypeptides as described previously (e.g., as a transgenic mouse).

15 *7. Screening of agents that delay, treat, or prevent arthritis*

In another embodiment, the invention provides a method of screening an agent to determine if the agent delays the onset of arthritis. The method includes: (a) providing a nonhuman mammal having a deficient NADPH oxidase pathway; (b) administering to the nonhuman mammal the agent; (c) inducing arthritis in the nonhuman mammal; and

20 (d) determining if the agent delays the onset of arthritis in the nonhuman mammal.

The mammal having a deficient NADPH oxidase pathway may be obtained as described previously. Determining if the agent delays the onset of arthritis may include steps such as: (a) determining a day of onset of arthritis value for the nonhuman mammal; and (b) comparing the day of onset of arthritis value for said nonhuman

25 mammal with a control day of onset of arthritis value. Onset of arthritis can be monitored using a macroscopic scoring system, wherein 1 point is given for each swollen or red toe, 1 point for each swollen midfood, digit, or knuckle, and 5 points for a swollen ankle, yielding a maximum score of 15 per limb and 60 total. The score can be a mean score, additive score, or maximum score. The mammal may be monitored 1 to 4 times a week

30 for 1 to 2 months after induction of arthritis. The control day of onset of arthritis value may be determined by determining a day of onset of arthritis value for a control

26

nonhuman mammal to which the agent has not been administered. The day of onset of arthritis in the nonhuman mammal may be considered delayed if it is later than the control day of onset value. The arthritis may be induced my conventional means and can be adjuvant induced arthritis, CIA, PIA, HIA, SIA, avridine induced arthritis, or OIA.

5   The invention also provides methods to screen an agent to determine if the agent treats arthritis. The method includes (a) providing a nonhuman mammal having a deficient NADPH oxidase pathway, where the nonhuman mammal exhibits symptoms of an arthritis (e.g., adjuvant induced arthritis, CIA, PIA, HIA, SIA, avridine induced arthritis, or OIA); (b) administering to the nonhuman mammal the agent; and (c)

10   determining if the agent treats arthritis in the nonhuman mammal. Such a determining step may involve (a) calculating an arthritis score in the nonhuman mammal; and (b) comparing the arthritis score with a control arthritis score. The mammal having a deficient NADPH oxidase pathway exhibiting symptoms of arthritis may be obtained as described previously. The control arthritis score may be determined by calculating an

15   arthritis score for a control nonhuman mammal to which the agent has not been administered. The arthritis score may be determined using the macroscopic scoring system described previously, and may be a mean score, additive score, or maximum score. The agent may be determined to treat arthritis if the arthritis score in the nonhuman animal is less than the control arthritis score.

20   In another embodiment, a method of screening an agent to determine if the agent prevents arthritis is provided. The method includes: (a) providing a nonhuman mammal having a deficient NADPH oxidase pathway; (b) administering to the nonhuman mammal the agent; (c) administering a compound known to induce arthritis to the nonhuman mammal; and (d) determining if the agent prevents arthritis induced by the

25   compound in the nonhuman mammal. Determining if the agent prevents arthritis may include evaluating said nonhuman mammal for symptoms of arthritis. Such an evaluation may occur for a period of time, e.g., for up to 20 days, up to 30 days, up to 50 days, or up to 70 days. Determining if the agent prevents arthritis may include comparing any symptoms of arthritis and their day of onset with the symptoms and day of onset of a

30   control nonhuman mammal to which said agent has not been administered. The macroscopic scoring system as described above may be used in the evaluation and

comparison. The compound known to induce arthritis may be an adjuvant, collagen, pristane, hexadecane, squalene, avridine, or oil. Collagen may be type II collagen; the oil may be incomplete Freund's adjuvant; and the adjuvant may be mycobaterial-derived.

The invention will be further described in the following examples, which do not

5   limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – Animals

Rat (*Rattus norvegicus*) strains used in the following experiments included the DA

10   strain, which is highly susceptible to PIA, and the E3 strain, which is PIA-resistant. DA and E3 rats were obtained from Zentralinstitut für Versuchstierzucht, Hannover, Germany, and were kept in animal facilities that have climate-controlled environments with 12-hour light/dark cycles. Rats were housed in polystyrene cages containing wood shavings and were fed standard rodent chow and water *ad libitum*. Rats were free from common

15   pathogens, including the Sendai virus, Hantaan virus, coronavirus, reovirus, cytomegalovirus, and *Mycoplasma pulmonis*. Animal breeding and experimentation were performed in the same animal facility.

Example 2 - Induction and Evaluation of Arthritis

20   Arthritis was induced in rats at the age of 8 to 12 weeks by intradermal injections of 150 µL of pristane (2,6,10,14-tetramethylpentadecane; Aldrich, Milwaukee, WI) at the bases of the tails. Arthritic development was monitored in all limbs using a macroscopic scoring system. Briefly, 1 point was given for each swollen or red toe, 1 point for each swollen midfoot, digit, or knuckle, and 5 points for a swollen ankle. The maximum score

25   for a limb was 15 points, and the maximum score for a rat was 60 points. Rats were examined from one to four times a week for one month after pristane injection.

For histopathologic analyses, at 31 days after pristane injections, rats were sacrificed, and the hind paws and ankle joints were prepared and analysed as follows. The paws were fixed in 4 % paraformaldehyde, decalcified in EDTA, embedded in

paraffin, and then sectioned and stained with hematoxylin and erythrosine as described in
Jonsson *et al.* (1986) *J Immunol Methods* 88:109-14.

Example 3 - Confirmation of Linkage to Arthritis Severity in Congenic Strains

5   A quantitative trait locus (QTL), denoted *Pia4,* was found to be associated with
PIA and CIA. To identify and isolate genes in the *Pia4* QTL that are associated with
arthritis, a 10 cM fragment of chromosome 12 from a PIA resistant E3 rat was
introgressed into a PIA susceptible DA rat resulting in a DA.E3chr12 congenic rat. Other
E3 genes on the 10 cM fragment that were not associated with RA were removed in
10   greater than 10 successive backcrosses with a DA rat. DA littermate rats obtained from
F2 intercrosses between DA.E3chr12 +/- rats were used as controls. The *Pia4* QTL was
inherited in a DA additive fashion with DA rats having the arthritis severity promoting
allele in the original F2 intercross between E3 and DA rats. Significant phenotypic
differences observed when a progeny rat is compared to the control rat could be
15   concluded to have arisen from genetic differences at the *Pia4* region.
            Arthritis was induced in 20 DA rats, 40 DA.E3chr12+/- rats, and 14 DA.E3chr12-
/- rats by injection with pristane, and the development of clinical arthritis was assessed as
described earlier. Figure 1 is a comparison of the clinical arthritis scores determined at
day 10, 14, 19, 24, and 28 after pristane injections of DA rats, DA.E3chr12+/- rats, and
20   DA.E3chr12-/- rats. Dramatic differences in arthritis severity were seen between DA
littermate controls and DA.E3chr12 congenic rats ($p < 0.0001$). The DA.E3chr12 -/-
congenic rats were still susceptible to arthritis, but the inflammation was very mild. The
incidences of arthritis observed at 31 days after pristane injections were 100 % among
DA rats, 58 % among DA.E3+/- rats, and 36 % among DA.E3-/- rats. When sections of
25   rat hind paw ankle joints obtained 31 days after pristane injections were stained with
hematoxylin and erythrosine and then examined microscopically at 100 X magnification,
cell infiltrations into the joints were observed. Although the PIA resistant E3 phenotype
afforded by the *Pia4* QTL was found to be almost dominant protective, DA progeny rats
carrying two E3 alleles of chromosome 12 exhibited even less severe arthritis ($p < 0.05$)
30   than DA progeny rats carrying one E3 allele of chromosome12.

The E3 derived fragment containing the *Pia4* QTL also was found to suppress CIA, HIA, and OIA (see Table 1). Other genes appear involved since E3 rats congenic for DA derived *Pia4*, (e.g. E3.DA chr12-/- rats) were still resistant to arthritis.

5   Table 1. Susceptibility of the *Pia4* congenic rat strain to CIA, OIA, and HIA. The P-value indicates the significance of the difference observed between *Pia4* congenic rats and DA rats. No significant difference between DA.E3chr12 +/- and DA.E3chr12 -/- was detected.

	Number of rats studied	Arthritis types	Maxscore	p-value
DA	10	OIA	15.1 ± 4.7	
DA.E3chr12 +/-	15	OIA	4.5 ± 1.6	<0.05
DA.E3chr12 -/-	15	OIA	1.2 ± 0.7	<0.05
DA	13	CIA	50.3 ± 3.4	
DA.E3chr12 +/-	16	CIA	29.8 ± 4.8	<0.01
DA.E3chr12 -/-	7	CIA	29.4 ± 6.0	<0.01
DA	9	HIA	14.6 ± 2.5	
DA.E3chr12 +/-	8	HIA	3.9 ± 2.4	<0.05

10   Example 4 - Sequencing and Physical Mapping

The P1-derived artificial chromosome (PAC) library of the BN rat (RPCI-31) described in Woon *et al.* (1998) *Genomics* 50:306-16 was obtained from the Resource Center of the German Human Genome Project (RZPD) (see world wide web at rzpd.de) as DNA pools and arrayed filters (library 712). Positive PAC clones also were obtained
15   from RZPD as well as clones that have DNA inserts corresponding to DNA in the vicinity of *Pia4* linked microsatellites (see Gosele *et al.* (2000) *Genomics* 69:287-94, world wide web at mdc-berlin.de/ratgenom, and world wide web at world wide web at

molgen.mpg.de/~ratgenome). PAC clones were purified using the Qiagen Large
Construction Kit (Qiagen, Hilden, Germany) and used for end sequencing with T7 and
SP6 primers as described in Woon et al. (1998) Genomics 50:306-16.

The rat *p47phox*, *GTF2i*, and *GTF2ird* cDNA sequences were determined as

5   follows. Publicly available sequences corresponding to rat *p47phox* (GenBank Accession
number AY029167), mouse *GTF2i* (GenBank Accession number AF017085) and mouse
GTF2ird (GenBank Accession number NM_020331) sequences were used for primer
design. E3, DA, and DA.E3chr12 RNAs were isolated using RNeasy Mini Kit (Qiagen,
Hilden, Germany). First strand cDNAs were synthesized from total RNA using the First-

10   strand cDNA Synthesis Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Double
stranded cDNA fragments were generated by conventional PCR and then ligated into the
pCR4-TOPO TA cloning vector. The resulting plasmid constructs were transformed into
competent *E. coli* (Invitrogen, Paisley, UK), and then purified from *E. coli* transformants
according to conventional alkaline lysis purification. Sequencing was performed using

15   the MegaBACE 1000 (Amersham Pharmacia Biotech, Uppsala, Sweden) sequencer and
the data obtained were analysed using Sequence Analysis 2.1 and SeqMan 4.05.

Example 5 - Genotyping and Statistic Analysis

DNA was prepared from toe biopsies and assayed with microsatellite markers by

20   polymerase chain reaction (PCR) analysed on MegaBACE 1000 (Amersham Pharmacia
Biotech, Uppsala, Sweden). Quantitative data were expressed as mean ± SEM, and
significance analysis was performed using nonparametric Mann-Whitney test.
Significance of frequency data was determined by Chi-square analyses.

25   ## Example 6 – Results of Physical Mapping and Positional Cloning

To identify genes within the *Pia4* QTL that contribute to the PIA resistant
phenotype in E3 rats, DA.E3chr12 congenic rats were backcrossed with DA rats, and a
large number of congenic progeny rats carrying overlapping *Pia4* fragments of different
sizes was used in the following experiment. Physical mapping was initiated with a

30   congenic fragment of 1 cM. Figure 2 is a physical map of the *Pia4* region constructed
using PAC clones and EST clones. Known microsatellites (d12rat72, d12got45,

d12got46 and d12rat26) in the *Pia4* region were used to identify PAC carrying inserts
corresponding to this region. Results of the Rat EST project at the University of Iowa
(see world wide web at ratEST.uiowa.edu) were used to identify EST clones known to
contain sequences in the *Pia4* region. The end sequences of the PAC clones and primer
5   sequences from EST clones were used in generating the physical map of the *Pia4* region.
In addition, sequence information from the *Pia4* region in rat (PAC clones RP31-78c13,
RP31-485f9, RP31-198l13, RP31-75g22, RP31-11m22, RP31-391d23 RP31-57j23), from
the partially sequenced BAC clones having inserts corresponding to the *Pia4* region (see
world wide web at hgsc.bcm.tmc.edu/projects/rat/), and sequences from homologous
10 regions in mouse (Genbank Accession numbers NT_029829, AF289665, AF139987 and
AF267747; see Kwitek *et al.* (2001) *Genome Res* 11:1935-43; Hoogenraad *et al.* (1998)
Genomics 53:348-58; Valero *et al.* (2000) *Genomics* 69:1-13) and human (Genbank
accession number NT_007867; see Peoples *et al.* (2000) *Am J Hum Genet* 66:47-68;
Osborne *et al.* (2001) *Nat Genet* 29:321-5) were assembled to form the *Pia4* physical
15 map.

The physical map of the *Pia4* region in combination with the arthritis
susceptibility of isolated congenic strains identified a minimal region of 300 kilobases
required for the PIA resistant phenotype. The 300 kilobase region contained two genes:
p47phox and *GTF2i*. The *p47phox* gene encodes Neutrophil Cytosolic Factor 1 (NCF1),
20 a subunit of the NADPH complex that produces oxygen radicals as a result of infection
(see Volpp *et al.* (1989) *Proc Natl Acad Sci U S A* 86:7195-9). The *GTF2i* gene encodes
Bruton Tyrosine Kinase (BTK)-Associated Protein (BAP-135), a substrate of BTK
involved in the B-cell receptor-signalling pathway, see Yang & Desiderio (1997) *Proc
Natl Acad Sci U S A* 94:604-9.

25

Example 7 – The *p47phox* E3 Allele has a Dominant Protective Role in Arthritis
Single nucleotide polymorphisms (SNPs) that distinguish the DA and E3 alleles in
the *Pia4* region were identified by sequencing cDNA using the Pyrosequencing
(Pyrosequencing, Uppsala, Sweden) according to protocols supplied by the manufacturer.
30 SNPs were found in the *p47phox*, *GTF2i*, *GTF2ird1* and *Cyln2* (AJ000485) genes.

A comparison of the DA *p47phox* cDNA and the E3 *p47phox* cDNA revealed three SNPs (see Figure 3). All three polymorphisms were base substitutions of which two were non-synonymous and resulted in substitutions at amino acid positions 106 and 153. These sequence polymorphisms included (1) substitution of an adenine with a guanine

5   nucleotide at position 330 in an E3 rat when compared a DA rat (DA/E3; A330G) resulting in replacement of a methionine residue with a valine residue at position 106 (Met106Val); (2) substitution of a thymine with a cytosine nucleotide at position 472 in an E3 rat (DA/E3; T472C) resulting in replacement of a methionine residue with a threonine residue at position 153 (Met153Thr); and (3) substitution of an adenine with a

10   cytosine at nucleotide 1161, a synonymous substitution that did not lead to an amino acid alteration. The sequence of the *p47phox* cDNA from the DA rat was identical to the published sequence of *p47phox* (AY029167) from Sprague-Dawley rat.

A comparison of the DA *GTF2i* cDNA sequence with the E3 *GTF2i* sequence revealed that the E3 sequence had a nucleotide substitution at position 2992. The

15   thymine nucleotide at position 2992 in the DA sequence was substituted with a cytosine nucleotide in the E3 sequence (DA/E3; T2992C). This substitution occurred in the untranslated region and so did not affect the GTF2i protein sequence.

Similar comparisons of the *Cyln2* and *GTF2ird1* genes of DA and E3 rats revealed SNPs at nucleotide 4206 (DA/E3: G4206A) in the *Cyln2* gene and at nucleotide

20   2823 (DA/E3: G2823C) in the *GTF2ird1* gene.

Example 8 - Prevalence of the *p47phox* Polymorphisms in Inbred and Wild Rats

The sequences of *p47phox* genes in other inbred rat strains and in wild rats were analysed for the presence of the three polymorphisms in the *p47phox* E3 allele (Table 2).

25   High degrees of polymorphisms in inbred rats as well as in wild rats were detected. This suggests that these polymorphisms did not result from domestification or from mutations generated in inbred laboratory rats. In fact, the DA and E3 alleles occurred in equal frequency indicating that these alleles were maintained by natural selection.

Table 2. Polymorphisms in the *p47phox* gene identified in DA/E3 inbred rat and wild rat populations.

Rat strain	SNP 330bp	SNP 472bp	SNP 1161bp	Rat strain	SNP 330bp	SNP 472bp	SNP 1161bp
DA	DA	DA	DA	SPRD-Cu3	DA	E3	DA
E3	E3	E3	E3	WAG-rnu	E3	E3	DA
ACI	E3	E3	E3	WC	E3	E3	DA
BDE	E3	E3	DA	WF	DA	DA	DA
BDII	E3	E3	DA	WKY	DA	DA	DA
BDIX	E3	E3	DA	KL-1	E3	E3	DA/E3
BDV	DA	DA	DA	KL-2	E3	DA/E3	DA/E3
BH	DA	E3	DA	KL-3	DA	DA	DA
BN	DA	E3	DA	KL-4	DA	DA	DA
BS	DA	E3	DA	KL-5	-	E3	-
COP	E3	E3	E3	KL-6	DA/E3	DA/E3	DA
DA-rnu	DA	DA	DA	KL-7	DA/E3	DA	DA
DXEA	E3	E3	E3	KL-8	DA/E3	DA	DA
DXEB	DA	DA	DA	KL-9	E3	DA	DA
DXEC	DA	DA	DA	KL-10	DA	DA	DA
DXER	DA	E3	DA	KL-11	E3	DA	E3
F344	DA	DA	DA	KL-12	DA	E3	-
GK	DA	DA	DA	KL-13	DA/E3	DA/E3	DA
LE	DA	DA	DA	KL-14	E3	DA	DA
LEW	DA	DA	DA	KL-15	E3	DA	DA
Lew (BM)	E3	E3	E3	KL-16	E3	DA	DA/E3
Lewis.1F	DA	DA	DA	KL-17	E3	DA	DA/E3
LOU7C	E3	E3	E3	KL-18	E3	DA	DA
LXB10	DA	DA	DA	JH-1	DA/E3	DA	DA/E3
LXB17L	DA	DA	DA	JH-2	DA	DA/E3	DA
LXB17N	DA	DA	DA	JH-3	DA	E3	DA
LXB19	DA	E3	DA	JH-4	DA	E3	DA
LXB21	DA	DA	DA	Ax-1	E3	DA	E3
LXB22	DA	DA	DA				
LXB24	DA	E3	DA				
LXB26	DA	E3	DA				
LXB27	DA	E3	DA				
LXB3	DA	E3	DA				
LXB30	DA	E3	DA				
LXB8	DA	DA	DA				
MNS	DA	E3	DA				
MWF	E3	E3	DA				
NAR	E3	E3	DA				
NEDH	DA	DA	DA				
NZNU	E3	E3	E3				
OM	DA	DA	DA				
PVG	E3	E3	E3				
RNU-rnu	E3	E3	E3				
SHR	E3	E3	E3				
SPRD	DA	DA/E3	DA				

A common haplotype present in BN rats included the DA polymorphism at nucleotide 330 and the E3 polymorphism at nucleotide position 472. Previously published crosses between DA and BN rats exhibiting disease conditions similar to multiple sclerosis (see Dahlman *et al.* (1999) *J Immunol* 162:2581-8) or arthritis (see

5   Griffiths *et al.* (2000) *Arthritis Rheum* 43:1278-89) revealed a locus with identical location as *Pia4* suggesting that disease protection is associated with threonine at amino acid 153. Since threonine is a potential phosphorylation site, and since the function of the human NADPH complex is highly regulated through phosphorylation of *p47phox*, it is likely that activity of P47PHOX and therefore activity of NADPH oxidase is affected by

10   the phosphorylation state of this residue. (See Faust *et al.* (1995) *J Clin Invest* 96:1499-505; El Benna *et al.* (1996) *J Biol Chem* 271:6374-8; Lal *et al.* (1999) *Biochem J* 338:359-66).

Example 9 - DA and DA.E3chr12 -/- Congenic Rats Exhibited Similar *p47phox*

15   Expression Levels

Expression of the *p47phox* gene in the spleens and lymph node tissues of DA and DA.E3chr12 rats was analysed by Northern blot hybridisation. Three DA and three DA.E3chr12-/- rats were subjected to pristane injection. Eight days after pristane injections, 10 μg of total RNA from the spleen and inguinal lymph node were isolated.

20   RNA was separated on an agarose/formaldehyde gel, transferred onto nylon membrane, and fixed by ultraviolet irradiation. The resulting Northern blot was subjected to hybridization with two probes: (1) α^{32}P-dCTP labeled DNA encoding rat P47PHOX and (2) α^{32}P-dCTP labeled DNA encoding 36B4-ribosomal protein. Hybridization was performed overnight at 42°C in Ambion ULTRA hybridization buffer (Ambion, Austin

25   Texas). After hybridization, the Northern blot was washed according to the manufacturer's instructions, and then subjected to phosphor imaging using the Kodak screen and Imager FX system (Bio-Rad, Hercules CA, USA). A probe directed against acidic ribosomal protein (Z29530) was used as the control for the total amount of RNA analysed.

30   No difference in the expression level of p47phox mRNA was detected when DA and DA.E3chr12 -/- congenic rats were compared. Therefore, polymorphisms in the

p47phox gene rather than differences in the expression level of *p47phox* accounted for the difference in arthritis severity.

Polypeptide expression of p47phox was analyzed by western blot. Spleen cells were lysed for 2 hours at 4°C in lysis buffer (3×10^7 cells/100 μL), 150 mM NaCl, 1 mM

5   CaCl$_2$, 1 mM MgCl$_2$, 20 mM Tris pH 7.4, 0.5% triton X-100 (Sigma), 0.25 M sucrose, protease inhibitor tablet (Roche Diagnostics), and 10 μg/mL deoxyribonuclease I (Sigma), and centrifuged at 14000 rpm. Equivalent amounts of total protein in the supernatant, determined by a protein assay kit (BioRad), were subjected to SDS-PAGE using a 10% separation gel. The polypeptides were electrophoretically transferred to a

10   nitrocellulose membrane (Biorad). The membranes were incubated with polyclonal rabbit antibodies to the Ncf1 peptide RRS TIR NAQ SIH QRC, biotinylated donkey anti-rabbit IgG (Jacksson Immunoresearch Laboratories), and ExtrAvidin peroxidase conjugate (Sigma). Immunoreactive polypeptide bands were visualized using enhanced chemoluminescence, ECL, reagents, and Hyperfilm (Amersham Pharmacia Biotech).

15   No difference in polypeptide expression between DA and DA.E3chr12 -/- rats was observed.

Example 10 - Plasma Levels of COMP and α_1-AGP Were Lower in DA.E3chr12

Congenic Rats than in Control Rats

20   Cartilage oligomeric matrix protein (COMP) and α_1-acid glycoprotein (AGP) are two plasma markers that are closely associated with RA as well as PIA. AGP, produced by hepatocytes, is a marker of general inflammatory response, while circulating COMP is the product of cartilage destruction and/or cartilage turnover. At 31 days after pristane injections, levels of AGP and COMP in the serum of DA, DA.E3chr12+/-, and

25   DA.E3chr12-/- rats were determined as follows.

The serum level of AGP was measured in a competitive radioimmunoassay (RIA; see Akerstrom (1985) *J Biol Chem* 260:4839-44) using a rat α_1-acid glycoprotein (Zivic-Miller Laboratories, Zelienople, PA) and a polyclonal anti-rabbit antibody specific for rat α_1-acid glycoprotein (Agrisera, Vännäs, Sweden).

30

Plasma concentration of COMP was determined by enzyme linked inhibition immunosorbent assay (ELISA) as described in Saxne & Heinegard (1992) *Br J Rheumatol* 31:583-91. Rat COMP was used for coating microtiter plates and for preparing standard curves. Plasma COMP was detected using a polyclonal antiserum

5   generated against rat COMP as the capturing antibody. The polyclonal antiserum was obtained from professor Dick Heinegård.

Figures 4A and 4B are bar graphs demonstrating that plasma levels of COMP and AGP in DA.E3chr12 congenic rats were significantly lower than plasma levels of COMP and AGP in DA controls ($p < 0.005$).

10

Example 11 - Oxidative Burst Assay Demonstrating That the Arthritis Severity-Promoting Variant of the *p47phox* Gene in DA Rats is Linked to Decreased Oxidative Burst

The function of P47PHOX was examined by assessing production of reactive

15   oxygen species (ROS) by peritoneal neutrophils. Rats were injected intraperitoneally with 5 mL of 2.4 % thioglycollate to elicit recruitment of neutrophils. Sixteen hours after thioglycollate injections, rats were sacrificed, and neutrophils were isolated from the peritoneal cavities as follows. The peritoneal cavities were rinsed with Hank's balanced salt solution (HBS), and the cells obtained were washed twice with HBS and then

20   resuspended at a concentration of 10^7 cells/mL.

The activity of the NADPH complex as indicated by production of ROS was determined by spectrophotometric detection of cytochrome C reduction (see Lomax *et al.* (1989) *Science* 245:409-12; Huang *et al.* (2000) *J Leukoc Biol* 67:210-5; and Zu *et al.* (1996) *Blood* 87:5287-96). Briefly, 5×10^6 cells were dispensed into each well of a 96

25   well microtitre plate containing 100 μg of cytochrome C (Sigma C3131) yielding a total volume 100 μL. Activation of NADPH complexes and secretion of superoxide radicals were initiated upon addition of PMA (Sigma P8139) to a concentration of 0.1 μg/mL. Reactions occurred at 37°C, and the absorbance at 550 nm was measured at 1-minute intervals.

30   Figure 5A-D is a set of absorbance curves illustrating production of ROS by DA rats, E3 rats, and *Pia4* congenic rats (DA.E3c12+/- and DA.E3c12-/- rats). Production of

oxygen radicals was determined from determining the area under the curve. These results
demonstrate that the production of ROS is higher in E3 and *Pia4* congenic rats than in
DA littermates. About three to six rats were examined in each genotypic group.

Unlike human patients with autosomal chronic granulomatous disease (CGD) (see
5   Casimir *et al.* (1991) *Proc Natl Acad Sci U S A* 88:2753-7) or mice deficient in functional
p47phox (see Jackson *et al.* (1995) *J Exp Med* 182:751-8; and Huang *et al.* (2000) *J
Leukoc Biol* 67:210-5), DA rats were not completely lacking in functional NADPH
complexes or ROS production. While E3 rats and DA.E3chr12 congenic rats exhibited
maximum cytochrome C reduction (OD_{550} = 0.2) 1000 seconds after PMA stimulation,
10   levels of ROS production in DA rats were slower and did not attain maximum
cytochrome C reduction even after 3000 seconds. Therefore, the arthritis severity-
promoting variant of the *p47phox* gene in DA rats is linked to decreased oxidative burst.

Example 12 – NAPDH Inhibition in DA.E3chr12 Rats

The following experiment was performed to determine if the protective effect of
15   the *Pia4* QTL is accounted for by the presence of polymorphisms in the *p47phox* gene
leading to decreased free radical production from NADPH complexes.

DA and DA.E3chr12 rats were given diphenyleneiodonium chloride (DPI,
$C_{12}H_8ICl$, MW = 314.6), a potent inhibitor of nitric oxide synthase (NOS) and
flavoenzymes such as NADPH oxidase. DPI had been used *in vivo* in mice to inhibit
20   potassium peroxochromate-induced arthritis (see, Miesel *et al.*, (1996) *Free Radic Biol
Med* 20:75-81). Daily intraperitoneally administration of 2.8 μmol/kg weight of a mouse
(i.e., 880 μg/kg) was found to reduced arthritis by 50 percent.

In this experiment, DPI was administered at a dosage of 1.25 μmol DPI/kg weight
of a rat (i.e., 0.4 mg/kg weight of a rat). Assuming that each rat had a mean weight of
25   225 g, about 0.08 mg of DPI in 0.5 mL of DMSO/PBS was given to each rat. For
administration, 50 mg of DPI was dissolved in 250 mL DMSO/PBS. About 0.5 mL was
injected intraperitoneally into rats on day 0, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 17, 19, and 21.

Arthritis was induced in all rats at the age of 8 to 12 weeks by intradermal
injections of 150 µL of pristane at the bases of the tails. Arthritis development was
30   monitored in all limbs. Results presented in Figure 6 and 7 reveal that inhibition of

NADPH oxidase with DPI lead to increased severity of arthritis. Therefore, NADPH oxidase activity has a protective role in development of PIA.

Example 13 - Transfer of ConA Activated T-cells from a DA Rat Induced Arthritis in E3 Rats and DA.E3chr12 Congenic Rats

To determine if the *Pia4* QTL controls the priming of arthritogenic T-cells or is involved in events that occur subsequent to the onset of arthritis, reciprocal spleen T-cell transfer experiments were performed using DA littermate controls, DA.E3chr12 +/- rats, and DA.E3chr12 -/- rats as donors or recipients of spleen cells activated with the T-cell mitogen ConA. Three rats from each group were used as donors, and three rats from each group were used as recipients.

Donor rats were injected with 500 μL of pristane. Twelve days after injections, rats were sacrificed, and their spleens were removed and homogenized for spleen T-cell isolation. Spleen cells were activated with ConA prior to transfer to recipient rats. Briefly, spleen T-cells were activated by culturing them at a cell density of 4×10^6 cells/mL for 48 hours at 37°C in DMEM medium (Paisley Scotland), supplemented with streptomycin, D-penicillin, HEPES, β-mercaptoethanol, 5 % fetal calf serum, and 3 μg/mL Con A. Spleen cells were then harvested, resuspended in PBS, and injected intraperitoneally into recipient rats at a concentration of 35×10^6 cells/animal.

Arthritis onset was detected at day 5 after T-cell transfer, and the highest arthritis scoring was obtained at day 15. Figure 8 is a bar graph illustrating that transfer of ConA activated T-cells from a DA rat gave rise to severe arthritis in E3 rats, in DA.E3chr12 +/- rats, and in DA.E3chr12 -/- rats. In addition, only ConA activated T-cells from a DA rat were arthritogenic. Therefore, the *p47phox* polymorphisms are involved in the generation of arthritogenic T-cells, but do not appear to operate significantly in the joints since the DA.E3chr12 congenic rats were susceptible to arthritis when subjected to DA T-cell transfers. Since P47PHOX functions in the spleen before T cell transfer, it is likely that, through its role in generating ROS, P47PHOX affects T-cell/antigen presenting cell interaction in the lymphoid organs.

Example 14 – Evaluation of Alkanes as NADPH Oxidase
Activators in the Oxidative Burst Assay

Saturated alkanes molecules C8-C19 (n-octane, nonane, undecane, dodecane,

tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, and

5   nonadecane) were tested for their ability to activate the NADPH complex as described

below. The unsaturated fatty acids C14:1-C24:1 (myristoleic acid, palmitoleic acid, oleic

acid, linoleic acid, linolenic acid, 11c-eicosenoic acid, arachidonic acid, erucic acid, and

neuronic acid) and squalenee (Sigma) were also tested. The oils were solubilized by

dilution in cyclodextrin (100 mM in PBS).

10

Isolation of peritoneal neutrophils and macrophages:

5 mL thioglycolate (2.4%) was injected intraperitoneally in E3 rats to elicit

recruitment of neutrophils 16 hours before sacrifice. 40 mL PBS+hepes (1%) was

injected into the peritoneum and the cell suspension extracted. Red blood cells were

15   lysed with 0.84% NH_4Cl, and WBC were washed in PBS and resuspended in PBS at a

concentration of about 10^7 cells/mL.

Culture of peritoneal neutrophils and macrophages:

Human myeloma cell line HL60 was cultured in Dulbeccos complete medium

20   with Hepes, 10% Fetal calf serum, and Penicillin–Streptomycin. HL60 cells

differentiated to neutrophil-macrophages six days after addition of 1.25% DMSO. The

cells were spun down at 1200 rpm for 5 minutes and then washed with PBS twice. The

cells were then resuspended in PBS to a concentration of about 10^7 cells/mL.

25   *Oxygen burst analysis through WST-1 assay:*

10 μL of each of the oils to be tested was added with 9 μL of WST1 to the wells of

a 96 well microtitre plate. 10^6 cells/mL (100 μL of 10^7cells/mL) were added to each well

and the color change measured in a spectrophotometer at 450 nm at 37° for 60 minutes

(one measurement/min).

30

Cytochrome C assay:

   10 μL each of the oils to be tested were added to the wells of a 96 well microtitre
plate. 50 μL of cytochrome C (4 mg/mL) and 5×10^6 cells/well (50 μL of 10^7 cells/mL)
were added to each well and the absorbance measured in a spectrophotometer at 550 nm
5 at 37°C for 60 minutes (one measurement/minute).

FACS based burst assay:

   Cells (2-5 x 10^5 well) are preincubated for 10 minutes at 37°C with 1 μM
dihydrorhodamine (DHR) 123 in a 96 well plate in total volume of 200 μL. 10 μL each of
10 the compounds (e.g., oils) to be tested are added to the wells and incubated a further 20
minutes at 37°C. Production of reactive oxidants is assayed as formation of the
fluorescent dye rhodamine 123 (RH) from dihydrorhodamine 123 (DHR) using flow
cytometry.

15 *Results*

   Saturated alkane molecules were tested with the oxidative burst assay to
determine activators of the NADPH complex. The assays were tested in both peritoneal
cells from E3 rat after thioglycolate recruitment of neutrophils (Figure 9A) and HL60
cells (Figure 9B). The WST-1 test indicates changes in the extracellular concentration of
20 reactive oxygen species (ROS). Both cell types demonstrates the same general trends.
For example, in all assays, undecane was a potent activator.

   The arthritis-inducing effects of the oils was examined by injecting the oils
intradermally into DA rats. The scoring shows that oils more than about 14 carbons long
induce arthritis, while shorter carbon chains do not induce arthritis (Figures 10A, 10B,
25 and 10C). Note that $p < 0.05$ for alkanes shorter than C15 compared to C19. All statistics
evaluated with student's t-test. N=4 for all groups.

Example 15 - Prevention and Ameliorative Treatment of Arthritis
with NADPH-Activating Oils

1. Treatment and/or prevention of pristane-induced arthritis with undecane in vivo.

5   Groups of DA rats were injected intradermally at the base of the tail with 200 µL
of the oils as indicated below:

Undecane day –10, Pristane day 0;

Olive oil day –10, Pristane day 0;

Undecane day –5, Pristane day 0;

10   Undecanol day –5, Pristane day 0;

Olive oil day –5, Pristane day 0;

Undecane day 5, Pristane day 0; and

Olive oil day 5, Pristane day 0.

The rats were scored every other day starting at day 11 according to the extended

15   scoring system where every red or swollen toe or mid foot scored one point, and a red and
swollen ankle scored five points, yielding a total of 15 points/limb and 60 points/rat.
Olive oil and undecanol were used as controls.

2. In vivo treatment of Hexadecane-induced arthritis with hexadecene

20   Groups of DA rats were injected i.d. at the base of the tail as follows:

Hexadecane 200 µL day 0;

Hexadecene 200 µL day 0;

Hexadecene 200 µL day –5, Hexadecane 200 µL day 0;

25   Olive oil 200 µL day –5, Hexadecane 200 µL day 0;

Hexadecene 200 µL day +5, Hexadecane 200 µL day 0; and

Olive oil day 200 µL +5, Hexadecane 200 µL day 0.

The rats were scored as indicated above. Olive oil was used as a control.

Results

Undecane was examined for its potential preventive effect on the development or severity of PIA. Rats were treated with undecane on day –10, day –5, or day +5, and undecanol on day –5 (as a control). All rats were injected with pristane on day 0 (Figure

5   11A). There was a significant difference between both the additive score (Figure 11B) and the maximum score (Figure 11C) for rats treated with undecane on day –5 and the rats that were treated with olive oil on day –5. There was also a significant difference for both the additive score (Figure 11B) and the maximum score (Figure 11C) between the treatment with undecane on day –5 and the treatment with undecanol on day –5. Note

10 also that the rats that were treated with undecane on day +5 had a slightly higher score than the rats that were treated with undecane on day –10 or day –5 before injection with pristane.

All statistics were evaluated with student's t-test. p<0.05 for the difference in both additive score and maximum score for undecane at day –5 and olive oil at day –5.

15 p=0.05 for the difference in additive score and p<0.05 for the difference in maximum score between undecane at day –5 and undecanol at day –5. N=6 for all groups except group 4 where N=12.

A similar experiment was also performed to evaluate the protective effect of hexadecene on arthritis-induction by hexadecane. Rats were treated with hexadecene

20 either on day –5, day +5, or were not treated with hexadecene. All rats were injected with hexadecane on day 0, except group 3. Olive oil was used as control. The arthritis scores demonstrate a significant difference between the arthritis-inducing capacities of the two substances (Figures 12A, 12B and 12C). Hexadecane induces arthritis while hexadecene does not. The difference in scores between the rats that were treated with hexadecene on

25 day –5 and day +5 and the control rats is also significant. None of the rats treated with hexadecene developed arthritis.

Groups 3 and 4 were injected only with either hexadecane or hexadecene. All statistics were evaluated with student's t-test. p<0.05 for the difference in both additive score and maximum score for treatment day –5 and day +5 against no treatment (group

30 4). p<0.05 for the difference in both additive score and maximum score between hexadecane and hexadecene. There is also a significant difference between the groups

43

that were injected with hexadecene and olive oil day +5 ($p<0.01$ in the additive score and $p<0.05$ in maximum score). N= 6 for all groups except group 3 and 4 where N=4.

Discussion

When oils were injected into the base of the tails of DA rats to determine their
5   arthritis - inducing capacities, alkanes longer than about 14 carbons induced arthritis,
while shorter ones did not. It is interesting to note that a comparison of the activity of the
oils in the oxygen burst studies and the arthritis induction studies indicates three possible
mechanisms for participation of the oils in the induction and/or treatment of arthritis. For
example, in the oxygen burst studies, undecane activated the NADPH complex, but did
10   not induce arthritis in the tail-injection arthritis studies, while hexadecane not only
activated the complex but also induced arthritis. A comparison of the two experiments
demonstrates that the hexa-, hepta-, and octa-decanes activate the NADPH complex
according to the WST-1 assay and also induce arthritis. Pentadecane (15C), on the other
hand, did not activate the NADPH complex, but did induce arthritis, suggesting a third
15   mechanism for participation in the induction of the disease.

A similar relationship has been seen with hexadecane and hexadecene, and
pristane and phytol, where hexadecane and pristane induce arthritis, while hexadecene
and phytol do not (Lorenzen (1999) *Scand. J. Immunol. 49:45-50*). All four substances
activate the NADPH complex. The difference between hexadecane and hexadecene is a
20   single carbon-carbon double-bond in hexadecene, while pristane and phytol differ by an
acidic group. While not being bound by any theory, it is possible that an increased
polarity facilitates the metabolism of the hexadecene and phytol, and therefore prevents
them from inducing arthritis. Molecules with lower polarity cannot be metabolised as
easily, and may remain longer in tissues, inducing an immune response.
25   We investigated the preventive capacities of both hexadecene and undecane.
None of the rats treated with hexadecene showed any symptoms of arthritis whether they
were injected with hexadecene at day −5 or day +5. Further, these experiments verify that
hexadecane induces arthritis, while hexadecene does not. Pre-treatment with undecane
may be more protective if done at a longer time point before induction of arthritis with
30   pristane. For example, treatment with undecane at day +5 did not show as significant a
preventive effect as at day −10 or at day -5.

44

Example 16 - *In vivo* Distribution of Alkane Oils

[1-14C] - Hexadecane and [1-14C] - Oleic acid were purchased from Amersham. The oils were injected intradermally at the base of the tail in 18 DA rats (200 μL). At day 10, the organs (lymph nodes, spleen, liver and kidney) of three rats from each group were

5   collected. The organs were weighed and homogenized in a total volume of 2 mL PBS and frozen. The same procedure was followed at day 20 and at day 30. 1 mL of the homogenised samples was then added to 10 mL Ready Safe (Beckman) and counted in a beta counter. The distribution of radioactive labelled hexadecane to the lymph nodes was also inspected at earlier time points (day 3, day 6, day 10, and day 13).

10

Results:

The distribution of hexadecane *in vivo* was examined by injecting radioactively-labeled hexadecane i.d. at the base of the tail in DA rats. Radioactively-labeled oleic acid was administered in the same manner. Scoring of the rats showed that only the rats

15   injected with hexadecane had arthritis (Figure 13). The arthritis was acute and had a symmetric involvement of both hind and front paws. Analyses of the different organs collected (LN, spleen, kidney and liver) in the β-counter showed that the oils were exclusively accumulated in the lymph nodes at all time points measured (Figure 14). Hexadecane seems to be distributed to the lymph nodes to a higher extent than oleic acid.

20   The distribution of the oils to the lymph nodes appeared to decrease with time with no increase in the other organs analyzed.

The distribution of hexadecane to the lymph nodes at earlier time points was also measured as described. An increase in the accumulation of hexadecane in the lymph nodes until day 10 was observed.

25   The distribution of radioactively-labeled hexadecane had previously been investigated, showing that 14C- labeled hexadecane is disseminated predominantly to the lymph nodes (Kleinau et al., (1995). *Int. J. Immunopharmac.*, 17(5):393-401.).

The experiments indicate that the hexadecane oil exerts a pro-arthritogenic effect within the lymph nodes. The injection of both radioactively- labeled hexadecane and

30   radio-labeled oleic acid suggests that oleic acid does not induce arthritis because it is not distributed to the lymph nodes to as high an extent as the hexadecane. Furthermore,

hexadecane slowly accumulated in the lymph nodes with a maximum at day 10, correlating with the normal onset of the disease. After day 10, the concentration of the hexadecane oil slowly decreased (Figure 15).

5   Example 17 – Treatment of Experimental Allergic Encephalomyelitis (EAE) *in vivo*
          Groups of DA rats were injected at the base of the tail with 200 μL phytol at day –
10, day –5, and day +5. Olive oil was used as a control. At day 0, all rats were treated
(immunized) with 200 μL SCH (DA spinal cord homogenate, to induce EAE) i.d. at the
base of the tail. The rats were then scored according to the following scale for 40 days:

10   0=Normal

                      1=Tail weakness

                      2=Tail paralysis

                      3=Tail paralysis and mild waddle

                      4=Tail paralysis and severe waddle

15   5=Tail paralysis and paralysis of one limb

                      6=Tail paralysis and paralysis of a pair of limbs

                      7=Tetra-paresis

                      8=Pre-morbid or dead.

          EAE began to appear around day 9. The results show a significant difference in
20   both additive score (Figure 16A) and maximum score (Figure 16B) between the rats
treated with phytol and the control rats at day –10 and at day +5 ($p<0.05$).

          All statistics were evaluated with students' T test. $p<0.05$ for the difference in
maximum score between treatment with phytol and treatment with olive oil at day –10
and day +5. Note that N= 6 for all groups.

25   In both EAE and PIA, the SCH to induce EAE and the pristane to induce PIA
were injected intradermally at the base of the tail. The disease onset generally occurred in
a few weeks. Potential activators of the NADPH complex may alter the proliferation of
auto reactive T-cells and reduce the incidence, rate of onset, and severity of the diseases.

Example 18 - Effect of Route of Administration of Phytol on Treatment of PIA

Groups of DA rats were treated with phytol according to one of the following treatment regimens at day –5 and at day +5. All rats were injected with pristane (200 μL) at day 0 intradermally at the base of the tail. Phytol was administered to groups of rats as

5   follows:

25 μL phytol intranasally (i.n.);

200 μL phytol intraperitoneally (i.p.);

200 μL phytol intradermally at the base of the tail (i.d.); and

1 mL phytol per os (p.o.).

10   The rats were scored from day 9 using the extended scoring system (as discussed previously).

The experiment demonstrated an ameliorating and preventive effect of i.d. administration. Intraperitoneal (i.p.) and intranasal (i.n.) routes of administration also demonstrated ameliorative and preventive effects. (See Figures 17A, 17B and 17C).

15   Note that the intranasal group was skewed by one rat that exhibited severe arthritis, while the three other rats in the group were protected.

Example 19 - Collagen Induced Arthritis in Ncf1-deficient Mice

Animals

20   Mice (B6.Cg-m +/+ Lepr (db), formerly known as C57BL/6J-m +/+ Lepr^{db}) deficient for Ncf1 because of a point mutation in the splice site for exon 8 (Huang et al., (2000) *J. Leukoc. Biol. 67:210-215.*) were purchased from the Jackson Laboratory (Maine, USA). The mice were backcrossed to B10.Q (originally from Professor Jan Klein, Tübingen, Germany) for two generations to yield the Q haplotype in MHC and to

25   lose the leptin receptor (lepr) defect. B10.Q Ncf1 +/- were intercrossed for the Collagen Induced Arthritis (CIA) experiments in order to obtain littermate control animals. All arthritis experiments were approved by local (Malmö/Lund, Sweden) ethical committee license M7-01.

Induction and evaluation of arthritis

Arthritis was induced in all mice at the age of 9-15 weeks by an intradermal injection at the base of the tail of 150 μg rat CII (collagen II) emulsified in complete Freunds adjuvant (CFA; Difco, Detroit, MI) at day 0. At day 35, the mice were given a

5   booster injection at the same location of 50 μg rat CII in Freunds incomplete adjuvant (IFA). Arthritis development was monitored in all four limbs using a macroscopic scoring system. Briefly, 1 point was given for each swollen or red toe, 1 point for each swollen midfoot, digit, or knuckle, and 5 points for a swollen ankle, yielding a maximum score per limb of 15 and 60 total. The mice were examined 1 to 4 times a week for 2

10   months after immunization. At day 40, serum was obtained through tail bleeding and kept at -20° C until assayed.

Determination of serum levels of COMP

Serum concentration of cartilage oligomeric matrix protein (COMP) was

15   determined using an enzyme linked inhibition immunosorbent assay (ELISA). Rat COMP was used to coat the microtitre plates and to prepare the standard curve for each plate. Plasma COMP was detected by using a polyclonal antiserum raised against rat COMP (generously provided by Professor Dick Heinegård) as capturing antibody.

20   *Antibody response*

Antibodies against rat cartilage in plasma were analysed with ELISA in 96 well plates (Costar, Cambridge, MA), coated overnight at 4°C with 50 μL/well of PBS containing 10μg/mL of rat collagen II. All washings were performed using Tris-buffered saline (NaCl 1.3M, Tris 0.1M, pH 7,4) containing 0.1% Tween20 (Tris/Tween). The

25   plasma was diluted in PBS/0.1%Tween and analyzed in duplicate. The amounts of bound IgG antibodies were estimated after incubation with a donkey anti-mouse IgG coupled to peroxidase (Jackson Immunoresearch, Westgrove, PA) and ABTS as substrate, followed by detection in a SpectraMax (Molecular Devices). The relative amount of antibodies in plasma was determined by comparison with a positive control of an anti collagen II

30   standard.

Results

In order to determine the role of defects or deficiencies of functional Ncf1 and/or
the NADPH oxidase complex on arthritis, the development of CIA (collagen induced
arthritis) in Ncf1 deficient B10.Q mice was investigated. Wild type B10.Q mice normally

5   develop a mild/medium-severe arthritis, with onset after booster immunization (Svensson
et al. (1998) *Clin. Exp. Immunol. 111:521-526*). The results indicate both an earlier onset
(Figure 19) and increased severity of arthritis (P< 0.001) (Figures 18A, 18B, 18C) in
Ncf1 deficient mice as compared to B10.Q mice. Mice heterozygous for the defect Ncf1
showed a milder arthritis with later onset than the homozygous deficient mice (P< 0.005).

10   The quantification of serum levels of COMP (Cartilage Oligomeric Matrix
Protein) is regarded as a measurement of the cartilage erosion of peripheral joints. Serum
COMP was highly elevated (P<0.0001) in the Ncf1 -/- mice as compared to Ncf1 +/- and
wild type controls (Figure 20). Furthermore, in the Ncf1 deficient mice there was a
strong antibody response against collagen II at day 40 after the first immunization (Figure

15   21).

Example 20 - Distribution in the Lymph Nodes of 14C-hexadecane After Injection of Hexadecene at day –5

An experiment was performed to determine if oils compete for available space in

20   the lymph nodes and if such a competition was the reason for the preventive effects of
pre-treatment with hexadecene and undecane. At day –5, DA rats were injected with one
of the following amounts of hexadecene: 0 μL, 50 μL, 100 μL, or 200 μL. At day 0, all
rats were injected intradermally with 14C-hexadecane at the base of the tail. At day 10,
the rats were sacrificed and the lymph nodes were collected and placed in PBS,

25   homogenized, and frozen. 1 mL of the homogenate was then transferred to Ready Safe
(Beckman) and analysed in a β-counter (Beckman).

The amount of arthritis inducing hexadecane in draining lymph nodes was not
affected by larger amounts of protective hexadecene. These results indicate that there is
no competition for space in the draining lymph nodes that is of importance for the

30   protective effect of the oil.

<u>Example 21 - CIA in rats treated with phytol</u>

DA rats were treated according to one of the following alternatives:

200 μL phytol intra-dermal at the base of the tail at day –10

200 μL phytol intra-dermal at the base of the tail at day –5

5   200 μL phytol intra-peritoneal at day -5 and day +5

control untreated

CIA was induced in all animals at day 0 by injecting 150 μL of collagen II

dissolved in 75 μL 0.1 M Acetic acid and emulsified in 75 μL IFA. The rats were scored

from day 10 using the extended scoring system (see above).

10   Both intra-peritoneal and intra-dermal treatment with phytol prevented

development of arthritis in the CIA rat model (Figure 22).

<u>Example 22 - Treatment of active PIA with phytol or undecane</u>

Groups of DA rats were injected with pristane at day 0. The pristane (150 μL)

15 was injected intra-dermal at the base of the tail. The rats were scored from day 9 using

the extended scoring system (see above). Then, the rats were treated at day 21 and at day

26 with one of the following alternatives:

200 μL phytol intra-peritoneal

200 μL phytol intra-dermal at the base of the tail

20   200 μL undecane intra-peritoneal

200 μL undecane intra-dermal at the base of the tail

control untreated

All the phytol and undecane treatments were effective against active arthritis in

the PIA rat model (Figures 23A, 23B).

25

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction

with the detailed description thereof, the foregoing description is intended to illustrate and

not limit the scope of the invention, which is defined by the scope of the appended claims.

30 Other aspects, advantages, and modifications are within the scope of the following

claims.

WHAT IS CLAIMED IS:

1.   A method for assessing a mammal's susceptibility to develop an autoimmune
condition, said method comprising:

(a) providing a blood or synovial fluid sample containing a cell from a mammal;

(b) determining the level of NADPH oxidase activity of said cell after contacting
said cell with an NADPH oxidase activator;

(c) determining whether or not said level is less than a control level of NADPH
oxidase activity, wherein said control level is the average amount of NADPH oxidase
activity of control cells from a population of mammals without said autoimmune
condition, and wherein said mammals without said autoimmune condition are from the
same species as said mammal; and

(d) identifying said mammal as being susceptible to develop said autoimmune
condition when said level is less than said control level.

2.   The method of claim 1, wherein said autoimmune condition is arthritis or multiple
sclerosis.

3.   The method of claim 1, wherein said NADPH oxidase activator is norfloxacin,
phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate,
lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane,
tridecane, tetradecane, hexadecane, hexadecene, heptadecane, octadecane, galectin 1, or
galectin 3.

4.   The method of claim 1, wherein said level of NADPH oxidase activity is
determined by measuring superoxide.

5.   The method of claim 1, wherein said level of NADPH oxidase activity is
determined by measuring reactive oxygen species.

6.   The method of claim 1, wherein said population comprises at least ten mammals.

7.   The method of claim 1, wherein step (c) comprises determining whether or not said level is between 5 and 75 percent less than said control level, and wherein step (d) comprises identifying said mammal as being susceptible to develop said autoimmune

5   condition when said level is between 5 and 75 percent less than said control level.

8.   The method of claim 1, wherein step (c) comprises determining whether or not said level is between 25 and 55 percent less than said control level, and wherein step (d) comprises identifying said mammal as being susceptible to develop said autoimmune

10   condition when said level is between 25 and 55 percent less than said control level.

9.   A method for assessing a mammal's susceptibility to develop an autoimmune condition, said method comprising:

        (a) providing a blood or synovial fluid sample from a mammal;

15   (b) determining the level of a blood or synovial fluid component that reflects NADPH oxidase activity;

        (c) determining whether or not said level is less than a control level, wherein said control level is the average amount of said component in control samples from a population of mammals without said autoimmune condition, and wherein said mammals

20   without said autoimmune condition are from the same species as said mammal; and

        (d) identifying said mammal as being susceptible to develop said autoimmune condition when said level is less than said control level.

10.   The method of claim 9, wherein said autoimmune condition is arthritis or multiple

25   sclerosis.

11.   The method of claim 9, wherein said component that reflects NADPH oxidase activity is malonic dialdehyde.

30   12.   A method for assessing a mammal's susceptibility to develop an autoimmune condition, said method comprising determining whether or not a mammal comprises a

genetic variant of the gene encoding a polypeptide that functions in the NADPH oxidase pathway, wherein the presence of said genetic variant indicates that said mammal is susceptible to develop said autoimmune condition.

5   13.   The method of claim 12, wherein said autoimmune condition is arthritis or multiple sclerosis.

14.   The method of claim 12, wherein said genetic variant encodes a mutant polypeptide.

10

15.   The method of claim 14, wherein said mutant polypeptide is a GP91PHOX polypeptide, P22PHOX polypeptide, P40PHOX polypeptide, P47PHOX polypeptide, or P67PHOX polypeptide.

15   16.   The method of claim 14, wherein said mutant polypeptide is a P47PHOX polypeptide.

17.   The method of claim 16, wherein said mammal is a human, and wherein said mutant P47PHOX polypeptide comprises the sequence set forth in SEQ ID NO:6 with at 20   least two amino acid substitutions.

18.   The method of claim 16, wherein said mutant P47PHOX polypeptide comprises an amino acid sequence having an amino acid residue other than valine at the position that aligns with position 106 of SEQ ID NO:4 or an amino acid residue other than 25   threonine at the position that aligns with position 153 of SEQ ID NO:4.

19.   The method of claim 12, wherein said genetic variant comprises a mutation in a regulatory sequence of said gene.

20.   A method for diagnosing an autoimmune condition accompanied by NADPH oxidase deficiency in a mammal having an autoimmune condition, said method comprising:

        (a) providing a sample comprising a cell from said mammal;

5   (b) determining the level of NADPH oxidase activity of said cell after contacting said cell with an NADPH oxidase activator;

        (c) determining whether or not said level is less than a control level of NADPH oxidase activity, wherein said control level is the average amount of NADPH oxidase activity of control cells from a population of mammals without said autoimmune

10   condition, and wherein said mammals without said autoimmune condition are from the same species as said mammal; and

        (d) identifying said mammal as having said autoimmune condition accompanied by NADPH oxidase deficiency when said level is less than said control level.

15   21.   The method of claim 20, wherein said autoimmune condition is arthritis or multiple sclerosis.

       22.   The method of claim 20, wherein said autoimmune condition accompanied by NADPH oxidase deficiency is AANOD.

20

       23.   The method of claim 20, wherein said NADPH oxidase activator is norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, hexadecene, heptadecane, octadecane, galectin 1, or

25   galectin 3.

       24.   The method of claim 20, wherein said level of NADPH oxidase activity is determined by measuring superoxide.

30   25.   The method of claim 20, wherein said level of NADPH oxidase activity is determined by measuring reactive oxygen species.

26.   The method of claim 20, wherein said population comprises at least ten mammals without said autoimmune condition.

5   27.   The method of claim 20, wherein step (c) comprises determining whether or not said level is between 5 and 75 percent less than said control level, and wherein step (d) comprises identifying said mammal as having said autoimmune condition accompanied by NADPH oxidase deficiency when said level is between 5 and 75 percent less than said control level.

10

28.   The method of claim 20, wherein step (c) comprises determining whether or not said level is between 25 and 55 percent less than said control level, and wherein step (d) comprises identifying said mammal as having said autoimmune condition accompanied by NADPH oxidase deficiency when said level is between 25 and 55 percent less than

15   said control level.

29.   A method for diagnosing an autoimmune condition accompanied by NADPH oxidase deficiency in a mammal having an autoimmune condition, said method comprising:

20   (a) providing a blood or synovial fluid sample from said mammal;

           (b) determining the level of a blood or synovial fluid component that reflects NADPH oxidase activity;

           (c) determining whether or not said level is less than a control level of NADPH oxidase activity, wherein said control level is the average amount of NADPH oxidase

25   activity of control cells from a population of mammals without said autoimmune condition, and wherein said mammals without said autoimmune condition are from the same species as said mammal; and

           (d) identifying said mammal as having said autoimmune condition accompanied by NADPH oxidase deficiency when said level is less than said control level.

30

30.   The method of claim 29, wherein said autoimmune condition is arthritis or
multiple sclerosis.

31.   The method of claim 29, wherein said autoimmune condition accompanied by
5   NADPH oxidase deficiency is AANOD.

32.   The method of claim 29, wherein said component that reflects NADPH oxidase
activity is malonic dialdehyde.

10   33.   A method for diagnosing an autoimmune condition accompanied by NADPH
oxidase deficiency in a mammal having an autoimmune condition, said method
comprising determining whether or not said mammal comprises a genetic variant of the
gene encoding a polypeptide that functions in the NADPH oxidase pathway, wherein the
presence of said genetic variant indicates that said mammal has said autoimmune
15   condition accompanied by NADPH oxidase deficiency.

34.   The method of claim 33, wherein said autoimmune condition is arthritis or
multiple sclerosis.

20   35.   The method of claim 33, wherein said autoimmune condition accompanied by
NADPH oxidase deficiency is AANOD.

36.   The method of claim 33, wherein said genetic variant encodes a mutant
polypeptide.
25

37.   The method of claim 36, wherein said mutant polypeptide is a GP91PHOX
polypeptide, P22PHOX polypeptide, P40PHOX polypeptide, P47PHOX polypeptide, or
P67PHOX polypeptide.

30   38.   The method of claim 36, wherein said mutant polypeptide is a P47PHOX
polypeptide.

39.   The method of claim 38, wherein said mammal is a human, and wherein said
mutant P47PHOX polypeptide comprises the sequence set forth in SEQ ID NO:6 with at
least two amino acid substitutions.

5

40.   The method of claim 38, wherein said mutant P47PHOX polypeptide comprises
an amino acid sequence having an amino acid residue other than valine at the position
that aligns with position 106 of SEQ ID NO:4 or an amino acid residue other than
threonine at the position that aligns with position 153 of SEQ ID NO:4.

10

41.   The method of claim 33, wherein said genetic variant comprises a mutation in a
regulatory sequence of said gene.

42.   A method for treating a mammal having an autoimmune condition accompanied

15   by NADPH oxidase deficiency, said method comprising administering, to said animal, an
agent that enhances NADPH oxidase activity.

43.   The method of claim 42, wherein said autoimmune condition is arthritis or
multiple sclerosis.

20

44.   The method of claim 42, wherein said autoimmune condition accompanied by
NADPH oxidase deficiency is AANOD.

45.   The method of claim 42, wherein said agent is norfloxacin, phosphatidic acid,

25   diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine,
fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane,
hexadecane, heptadecane, octadecane, galectin 1, or galectin 3.

46.   The method of claim 42, wherein said agent is polar derivative of norfloxacin,

30   phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate,

lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane, galectin 1, or galectin 3.

47.   The method of claim 42, wherein said agent is an alkene or acid derivative of
5   norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane, galectin 1, or galectin 3.

10   48.   The method of claim 42, wherein said agent is administered intra-dermally, intra-peritoneally, or intra-nasally.

49.   The use of an agent in the manufacture of a medicament to treat an autoimmune condition accompanied by NADPH oxidase deficiency, wherein said agent enhances
15   NADPH oxidase activity in a mammal.

50.   The use of claim 49, wherein said autoimmune condition accompanied by NADPH oxidase deficiency is AANOD.

20   51.   The use of claim 49, wherein said autoimmune condition accompanied by NADPH oxidase deficiency is multiple sclerosis accompanied by NADPH oxidase deficiency.

52.   The use of claim 49, wherein said agent is norfloxacin, phosphatidic acid,
25   diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, hexadecene, heptadecane, octadecane, galectin 1, or galectin 3.

53.   A method of formulating a medicament for the treatment of an autoimmune
30   condition, said method comprising:

(a) contacting a sample comprising cells or a cellular fraction having NADPH activity with a test agent,

(b) determining the level of NADPH oxidase activity in said sample,

(c) determining whether or not said level is greater than a control level of NADPH
5   oxidase activity, wherein said control level is the amount of NADPH oxidase activity in a control sample lacking said test agent,

(d) identifying said test agent as an agent useful for treatment of said autoimmune condition when said level of NADPH oxidase activity is greater than said control level, and

10   (e) formulating a medicament from said agent for the treatment of said autoimmune condition.

54.   The method of claim 53, wherein said autoimmune condition is arthritis or multiple sclerosis.

15

55.   A method for identifying an agent that activates NADPH oxidase activity in a cell, wherein said cell is from a non-human animal susceptible to arthritis induction, said method comprising determining whether or not the level of NADPH oxidase activity increases in said cell after said cell is treated with a test agent, wherein an increase in said
20   level indicates that said test agent activates NADPH oxidase activity.

56.   The method of claim 55, wherein said cell is a cell from a DA rat.

57.   The method of claim 55, wherein said cell is a lymphocyte.

25

58.   The method of claim 55, wherein said non-human animal is susceptible to pristane induced arthritis, collagen induced arthritis, adjuvant induced arthritis, oil induced arthritis, hexadecane induced arthritis, or avridine induced arthritis.

30   59.   The method of claim 55, wherein said level of NADPH oxidase activity is determined by measuring superoxide.

59

60.   The method of claim 55, wherein said level of NADPH oxidase activity is determined by measuring reactive oxygen species.

5   61.   The method of claim 55, wherein said level of NADPH oxidase activity is determined with a cytochrome C assay or WST-1 assay.

62.   A method for identifying an agent useful in the treatment of an autoimmune condition, said method comprising:

10   (a) contacting a sample comprising cells or a cellular fraction having NADPH activity with a test agent,

     (b) determining the level of NADPH oxidase activity in said sample,

     (c) determining whether or not said level is greater than a control level of NADPH oxidase activity, wherein said control level is the amount of NADPH oxidase activity in a

15   control sample lacking said test agent, and

     (d) identifying said test agent as an agent useful for treatment of said autoimmune condition when said level of NADPH oxidase activity is greater than said control level.

63.   The method of claim 62, wherein said autoimmune condition is arthritis or

20   multiple sclerosis.

64.   A method for identifying an agent useful in the treatment of an autoimmune condition, said method comprising:

     (a) contacting a sample comprising cells or a cellular fraction having NADPH

25   activity with a test agent,

     (b) contacting said sample with an NADPH oxidase activator,

     (c) determining the level of NADPH oxidase activity in said sample,

     (d) determining whether or not said level is greater than a control level of NADPH oxidase activity, wherein said control level is the amount of NADPH oxidase activity in a

30   control sample treated with said activator in the absence of said test agent, and

(e) identifying said test agent as an agent useful for treatment of said autoimmune condition when said level of NADPH oxidase activity is greater than said control level.

65.   The method of claim 64, wherein said autoimmune condition is arthritis or
5   multiple sclerosis.

66.   A method for identifying an agent that enhances NADPH oxidase activity in a cell, wherein said cell is from a non-human animal susceptible to arthritis induction, said method comprising:
10   (a) contacting said cell with an NADPH oxidase activator and a test agent to form a test cell,
            (b) determining the level of NADPH oxidase activity in said test cell,
            (c) determining whether or not said level is greater than a control level of NADPH oxidase activity, wherein said control level is the amount of NADPH oxidase
15   activity in a control cell treated with said activator in the absence of said test agent, and
            (d) identifying said agent as enhancing NADPH oxidase activity when said level is greater than said control level.

67.   The method of claim 66, wherein said cell is a cell from a DA rat.
20
68.   The method of claim 66, wherein said cell is a lymphocyte.

69.   The method of claim 66, wherein said non-human animal is susceptible to pristane induced arthritis, collagen induced arthritis, adjuvant induced arthritis, oil induced
25   arthritis, hexadecane induced arthritis, or avridine induced arthritis.

70.   The method of claim 66, wherein said NADPH oxidase activator is norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate, lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane,
30   tridecane, tetradecane, hexadecane, heptadecane, octadecane, galectin 1, or galectin 3.

71.   The method of claim 66, wherein said level of NADPH oxidase activity is determined by measuring superoxide.

72.   The method of claim 66, wherein said level of NADPH oxidase activity is
5   determined by measuring reactive oxygen species.

73.   The method of claim 66, wherein said level of NADPH oxidase activity is determined with a cytochrome C assay or WST-1 assay.

10   74.   A method for identifying an agent that inhibits NADPH oxidase activity in a cell, wherein said cell is from a non-human animal susceptible to arthritis induction, said method comprising:

        (a) contacting said cell with an NADPH oxidase activator and a test agent to form a test cell,
15   (b) determining the level of NADPH oxidase activity in said test cell,

        (c) determining whether or not said level is less than a control level of NADPH oxidase activity, wherein said control level is the amount of NADPH oxidase activity in a control cell treated with said activator in the absence of said test agent, and

        (d) identifying said agent as inhibiting NADPH oxidase activity when said level
20   is less than said control level.

75.   The method of claim 74, wherein said cell is a cell from a DA rat.

76.   The method of claim 74, wherein said cell is a lymphocyte.
25

77.   The method of claim 74, wherein said non-human animal is susceptible to pristane induced arthritis, collagen induced arthritis, adjuvant induced arthritis, oil induced arthritis, hexadecane induced arthritis, or avridine induced arthritis.

30   78.   The method of claim 74, wherein said NADPH oxidase activator is norfloxacin, phosphatidic acid, diacylglycerol, arachidonic acid, phorbol myristate acetate,

lysophosphatidylcholine, fMLP, pristane, phytol, octane, decane, undecane, dodecane, tridecane, tetradecane, hexadecane, heptadecane, octadecane, galectin 1, or galectin 3.

79.   The method of claim 74, wherein said level of NADPH oxidase activity is determined by measuring superoxide.

80.   The method of claim 74, wherein said level of NADPH oxidase activity is determined by measuring reactive oxygen species.

81.   A method for identifying an agent that modulates T-cell activation, said method comprising:

    (a) determining whether or not a test agent increases NADPH oxidase activity; and

    (b) classifying said test agent as an agent that modulates T-cell activation when said test agent increases NADPH oxidase activity.

82.   The method of claim 81, wherein said modulation of T-cell activation is a reduction in T-cell activation.

83.   A rat congenic to a second rat, wherein at least one locus differs genetically between said rat and said second rat, wherein said second rat is susceptible to arthritis induction, wherein said rat contains T-cells from said second rat, and wherein said rat has arthritis.

84.   The rat of claim 83, wherein said rat is a DA.E3c12-/- rat.

85.   The rat of claim 83, wherein said second rat ia a DA rat.

86.   The rat of claim 83, wherein said at least one locus comprises nucleic acid that encodes a P47PHOX polypeptide.

87.   The rat of claim 83, wherein no more than one locus differs genetically between said rat and said second rat.

88.   The rat of claim 87, wherein said one locus comprises nucleic acid that encodes a P47PHOX polypeptide.

89.   A nonhuman mammal comprising a deficient NADPH oxidase pathway, wherein said nonhuman mammal exhibits symptoms of an autoimmune disease.

90.   A method of screening an agent to determine if said agent delays the onset of arthritis, said method comprising:
          (a) providing a nonhuman mammal having a deficient NADPH oxidase pathway;
          (b) administering said agent to said nonhuman mammal;
          (c) inducing arthritis in said nonhuman mammal; and
          (d) determining if said agent delays the onset of arthritis in said nonhuman mammal.

91.   A method for screening an agent to determine if said agent treats arthritis, said method comprising:
          (a) providing a nonhuman mammal having a deficient NADPH oxidase pathway, wherein said nonhuman mammal exhibits symptoms of an arthritis;
          (b) administering to said nonhuman mammal said agent; and
          (c) determining if said agent treats arthritis in said nonhuman mammal.

92.   A method of screening an agent to determine if said agent prevents arthritis, wherein said method comprises:
          (a) providing a nonhuman mammal having a deficient NADPH oxidase pathway;
          (b) administering to said nonhuman mammal said agent;
          (c) administering a compound that induces arthritis to said nonhuman mammal; and

64

(d) determining if said agent prevents arthritis induced by said compound in said nonhuman mammal.

93.   A method for identifying an agent that inhibits NADPH oxidase activity in a
lymphocyte, wherein said lymphocyte is from a DA rat, said method comprising:

(a) contacting said lymphocyte with PMA and a test agent to form a test cell;

(b) determining the level of NADPH oxidase activity in said test cell by measuring superoxide;

(c) determining whether or not said level is less than a control level of NADPH oxidase activity, wherein said control level is the amount of NADPH oxidase activity in a control cell treated with said PMA in the absence of said test agent; and

(d) identifying said agent as inhibiting NADPH oxidase activity when said level is less than said control level.

94.   A non-DA rat comprising heterologous T-cells from a non-human animal susceptible to arthritis induction, wherein said non-DA rat has arthritis.

95.   The rat of claim 94, wherein said rat is an E3 rat.

96.   The rat of claim 94, wherein said non-human animal is a DA rat.

Figure 1

Clinical scoring of PIA4 congenic rats
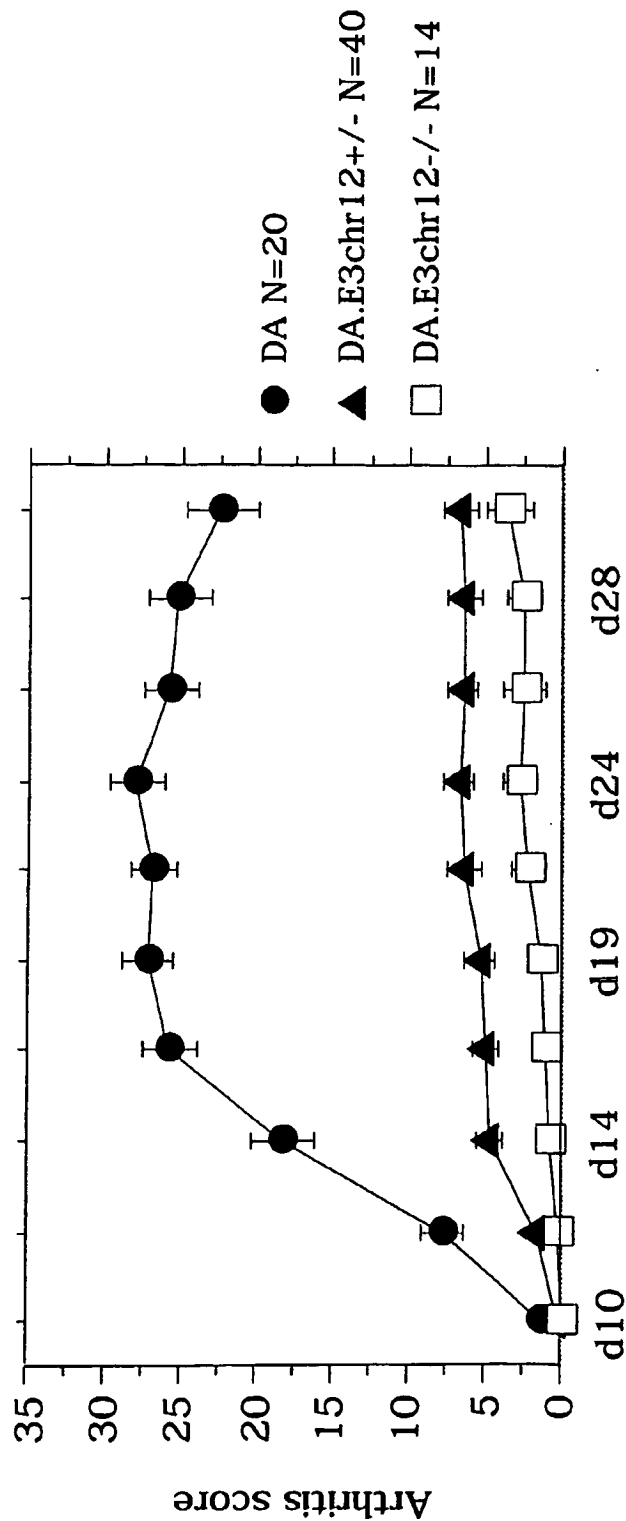
Incidence d31: DA (100%), DA.E3+/- (58%) and DA.E3-/- (36%)

Figure 2

Physical map of the Pia4 region
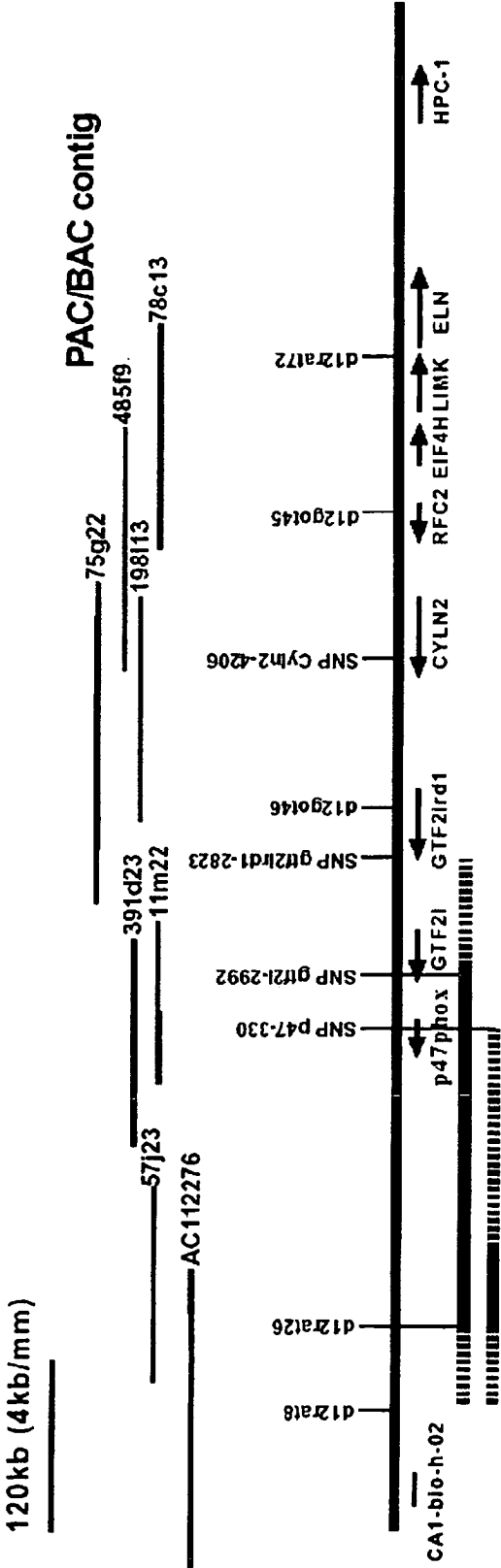
Figure 3

cDNA sequence p47phox
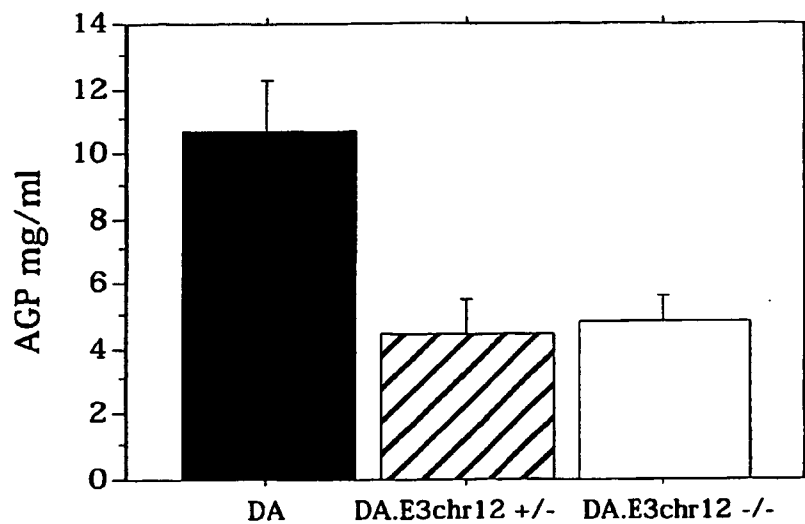
4/25

Figure 4A

COMP in DA.E3c12 rats
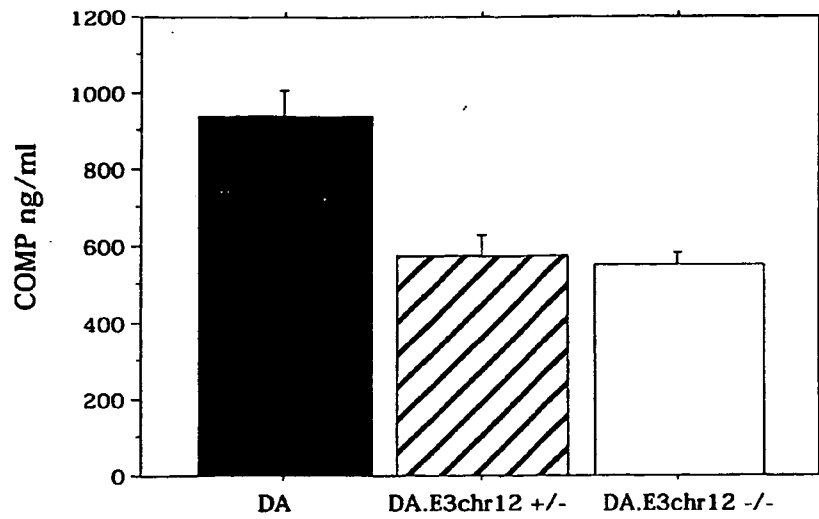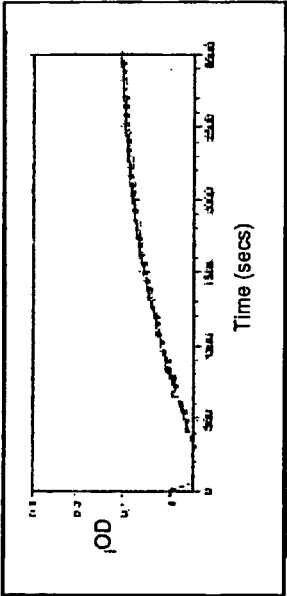

Figure 4B
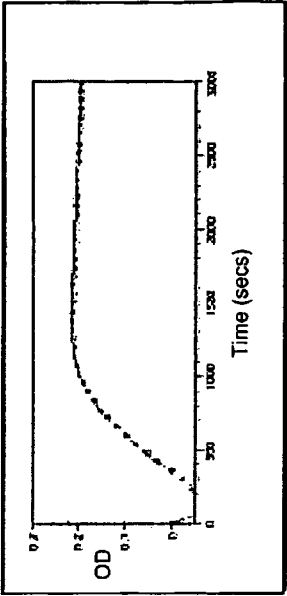
AGP in DA.E3c12 rats
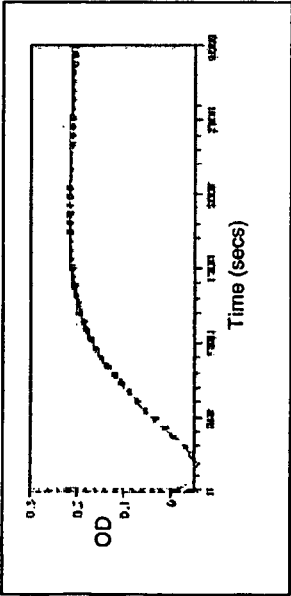
Figure 5A
DA

Figure 5B
E3

Figure 5C
DA.E3c12 +/-

Figure 5D
DA.E3c12 -/-
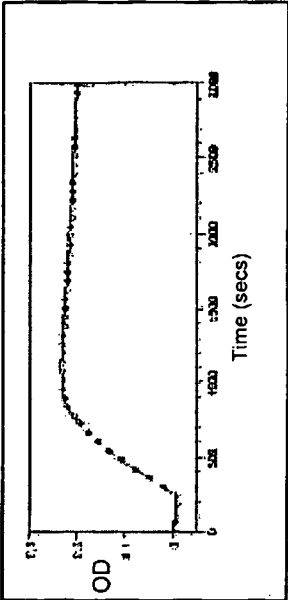
Figure 6

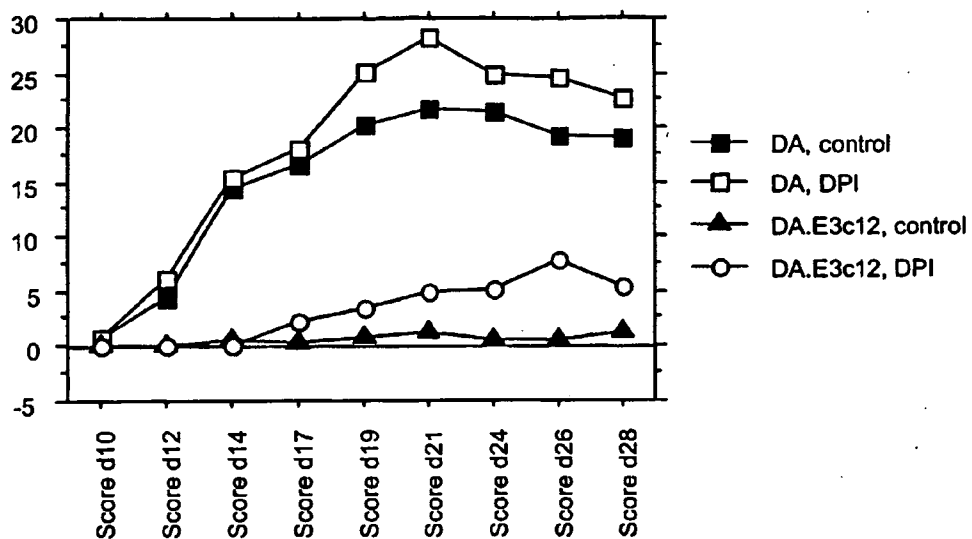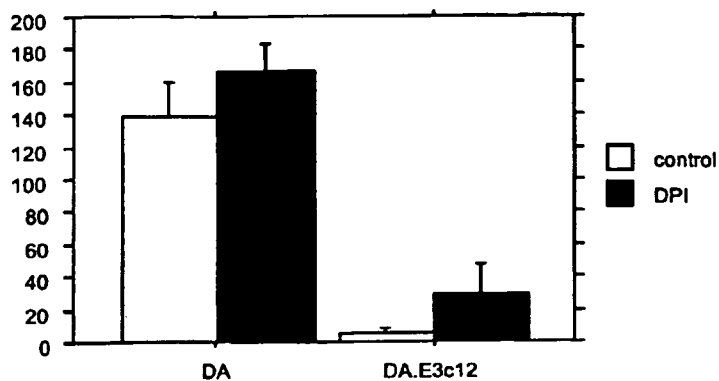**Pristane injected rats with NADPH inhibitor DPI injected i.p.**

Legend:
- ■ DA, control
- □ DA, DPI
- ▲ DA.E3c12, control
- ○ DA.E3c12, DPI

Figure 7

Accumulated arthritis score
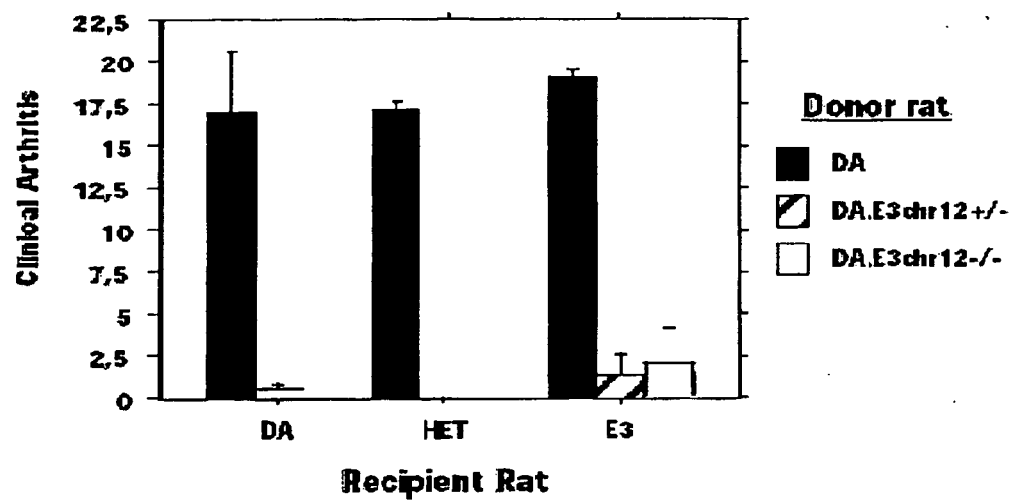

Legend:
- □ control
- ■ DPI

Figure 8

Transfer of pristane/ConA activated T-cells

Clinical scoring day 15 after T-cell transfer

Figure 9A

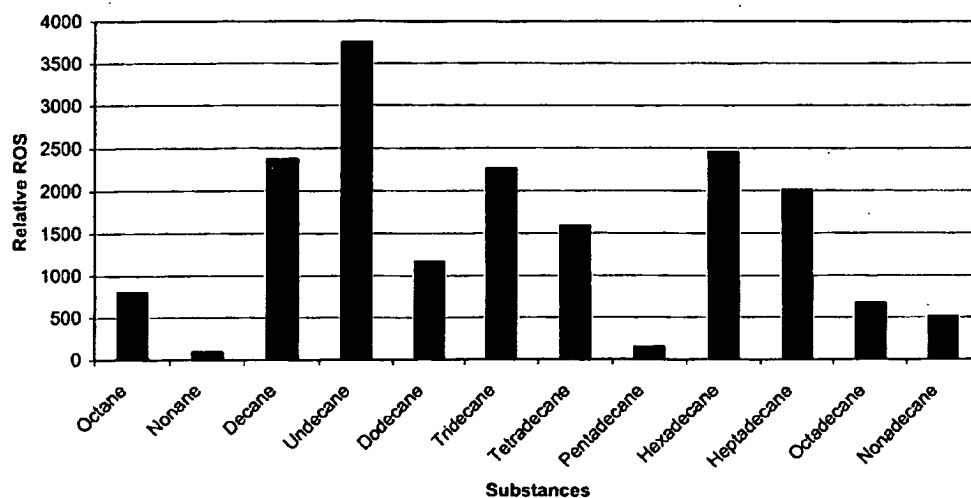**Activation of the oxidative burst by alkanes in vitro (E3 rat cells)**

Figure 9B

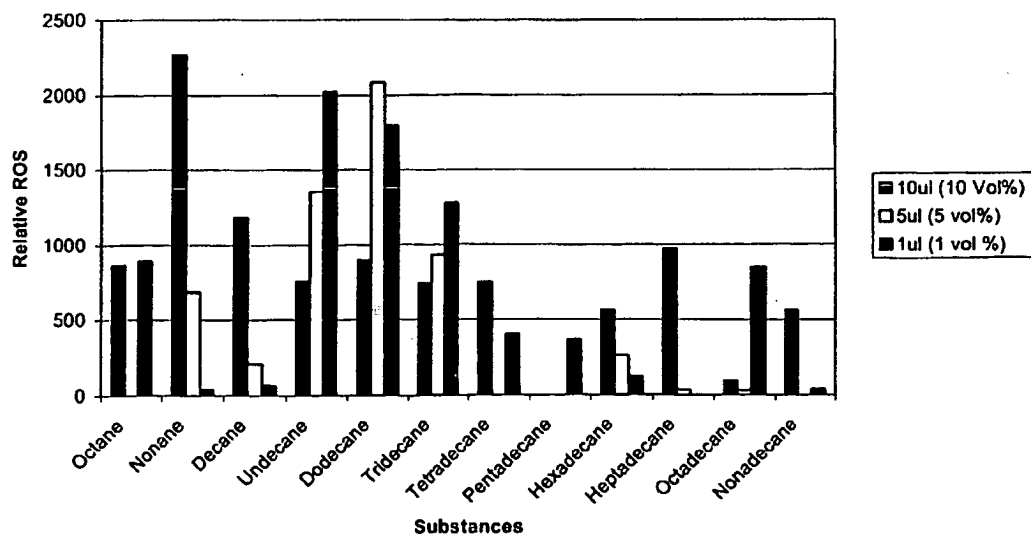**Activation of the oxidative burst by alkanes in vitro (HL60 cells)**

Figure 10A
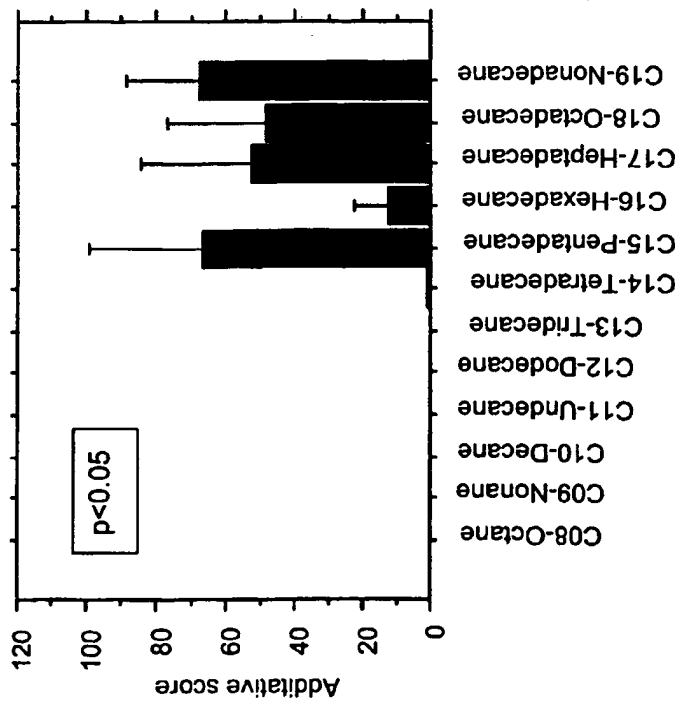

Arthritis inducing effects of alkanes

Figure 10C

Arthritis inducing effects of alkanes

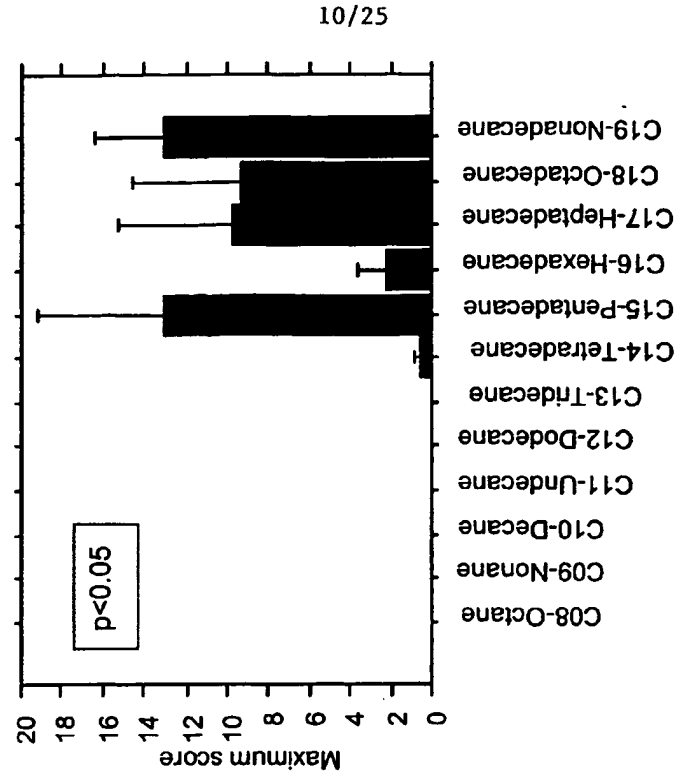# Figure 10B

Arthritis inducing effects of alkanes

Figure 11A

PIA in DA rats treated with undecane or undecanol

Figure 11C

PIA in DA rats treated with undecane or undecanol
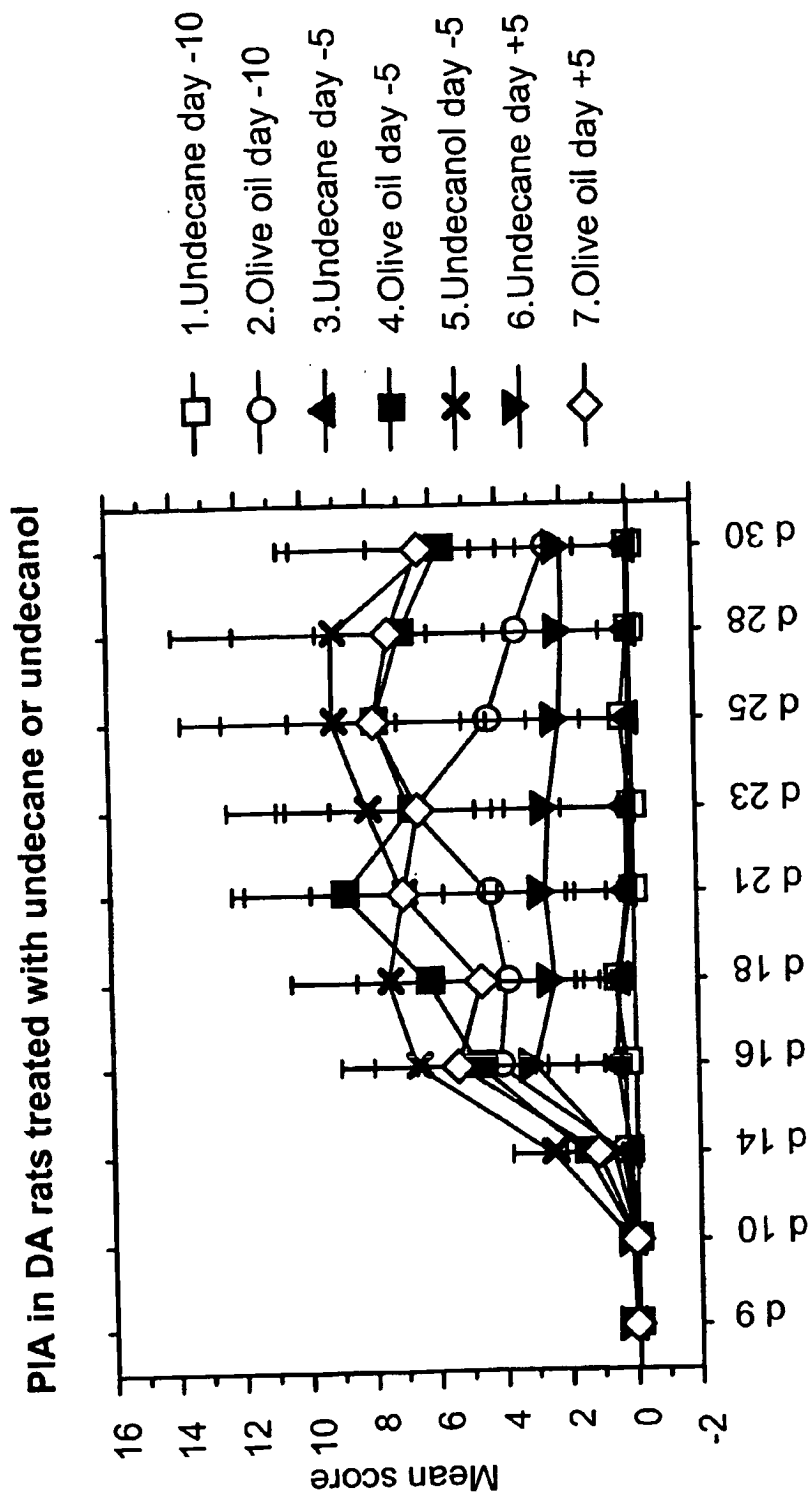
Figure 11B

PIA in DA rats treated with undecane or undecanol
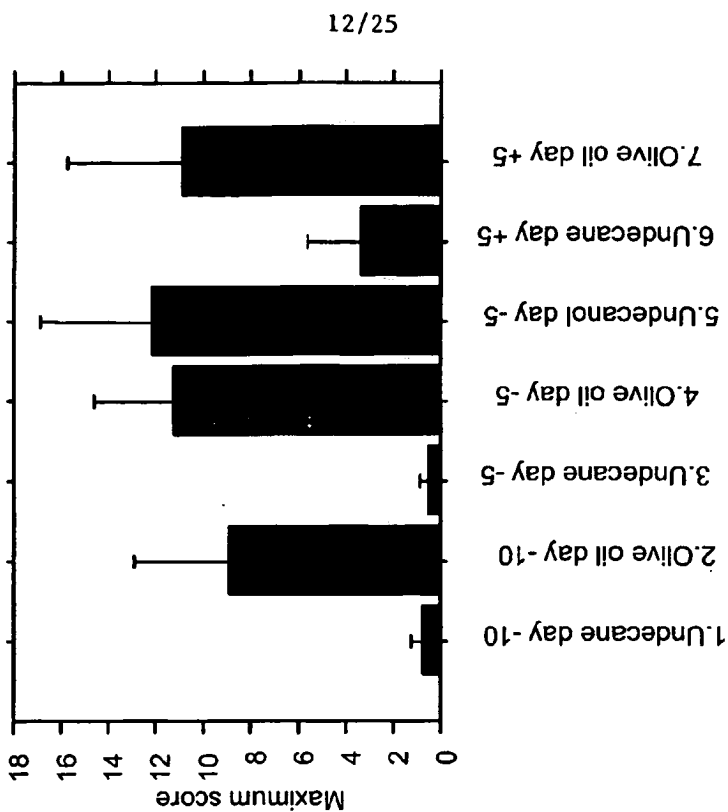
Figure 12A

Hexadecane induced arthritis after treatment with hexadecene

Figure 12C

Hexadecane induced arthritis after treatment with hexadecene
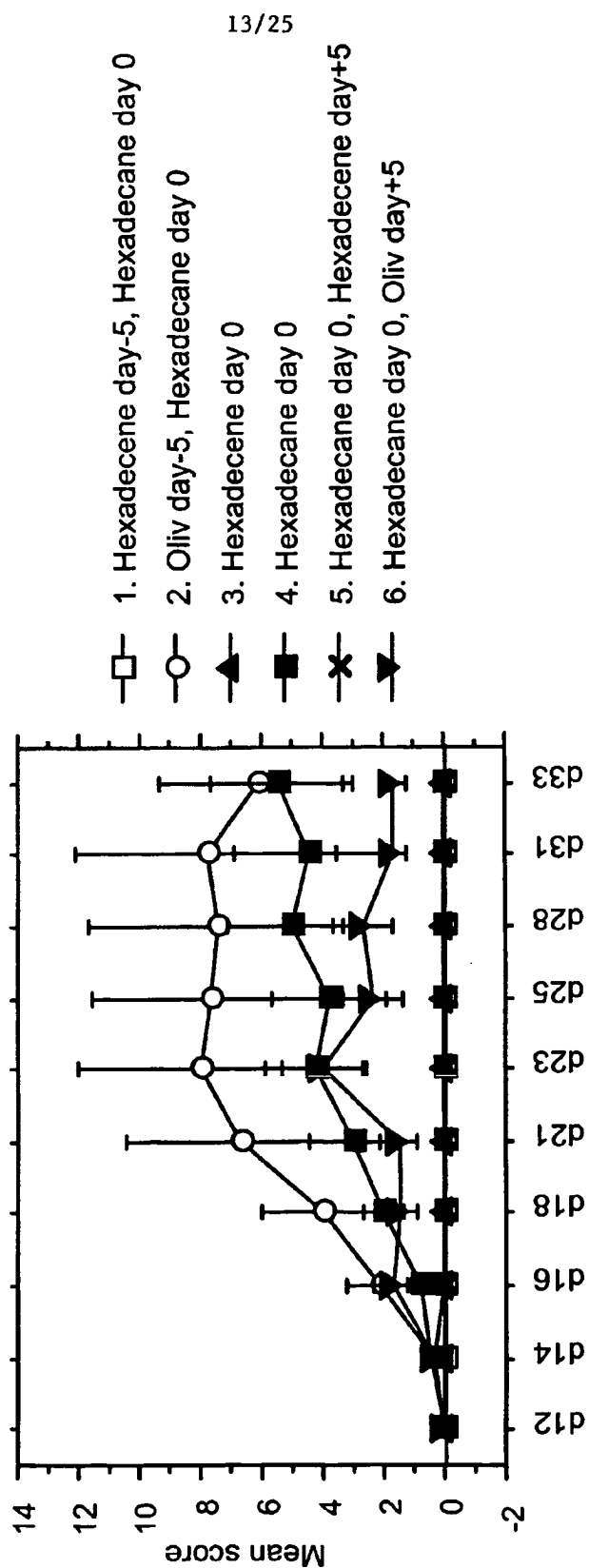
Figure 12B

Hexadecane induced arthritis after treatment with hexadecene
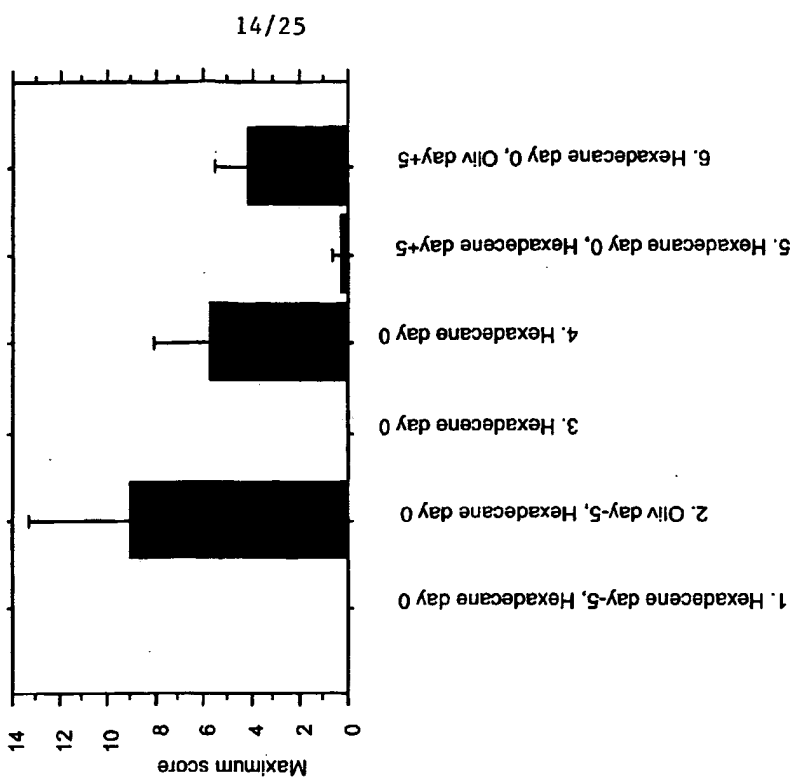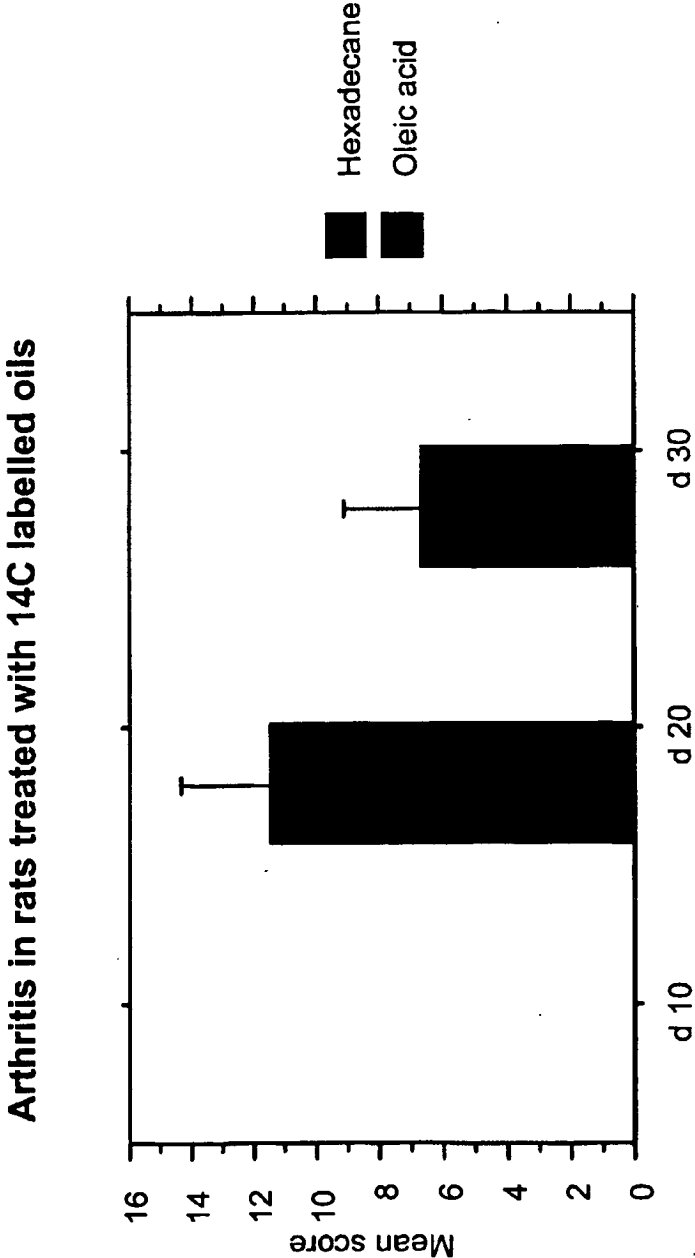
Figure 13

Figure 14

Tissue distribution of 14C labelled oils
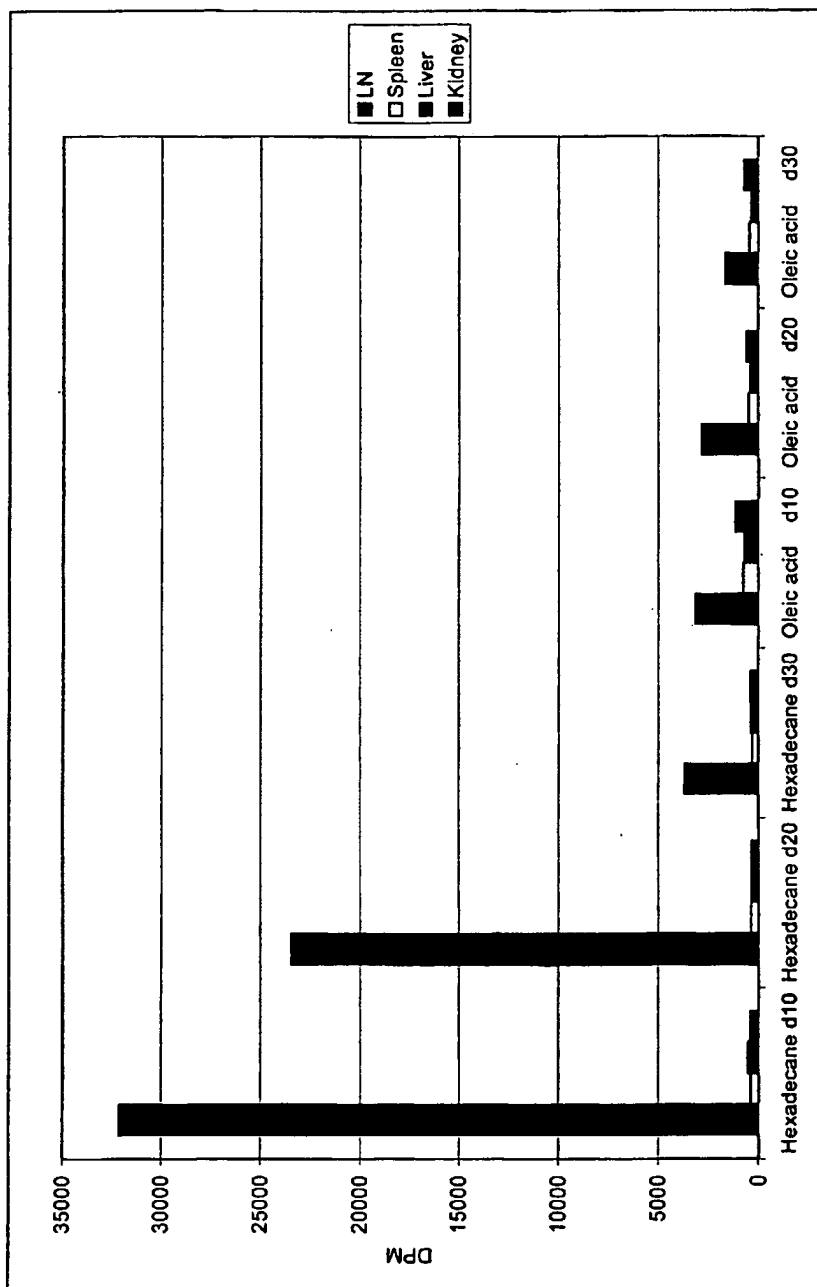
Figure 15

Figure 16B

Treatment of EAE in DA rats with phytol

Figure 16A

Treatment of EAE in DA rats with phytol

Figure 17A

Effect of treatment of PIA using phytol with different routs of administration

Figure 17C

Effect of treatment of PIA using phytol
with different routs of administration
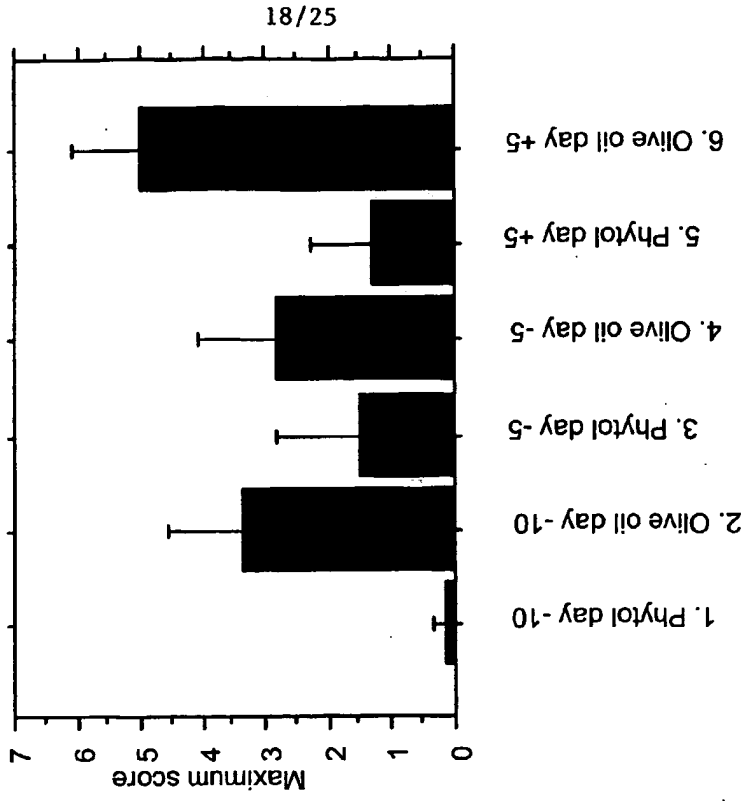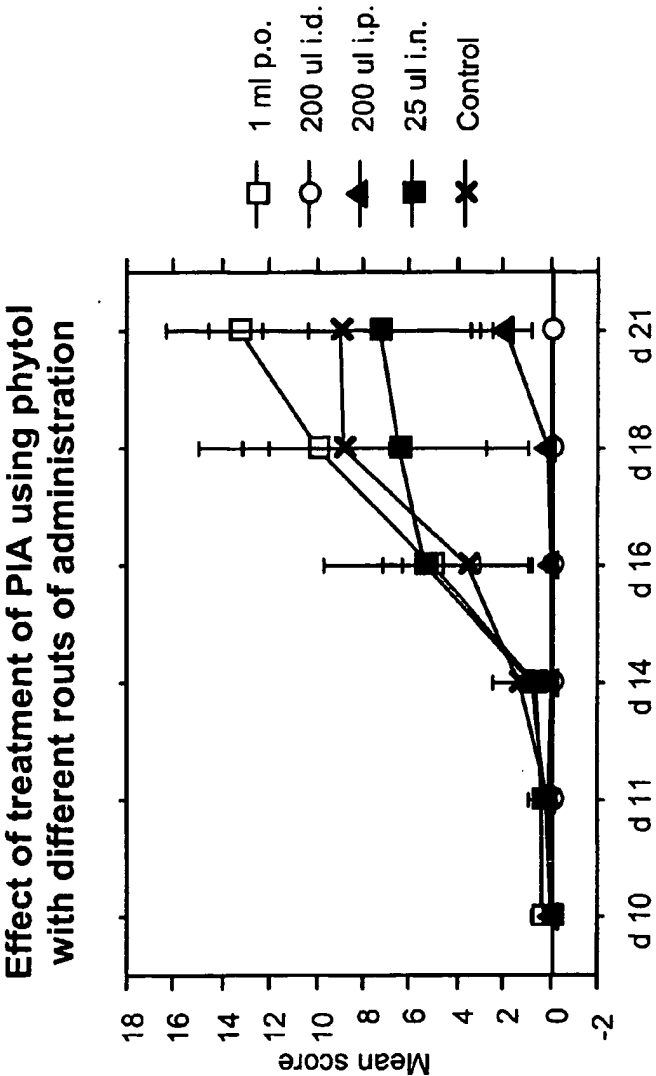
Figure 17B

Effect of treatment of PIA using phytol
with different routs of administration
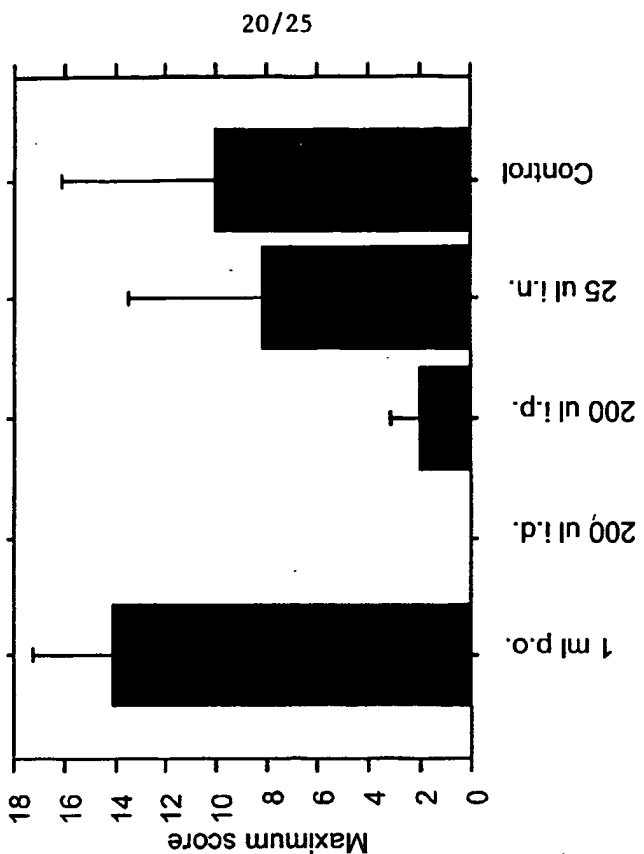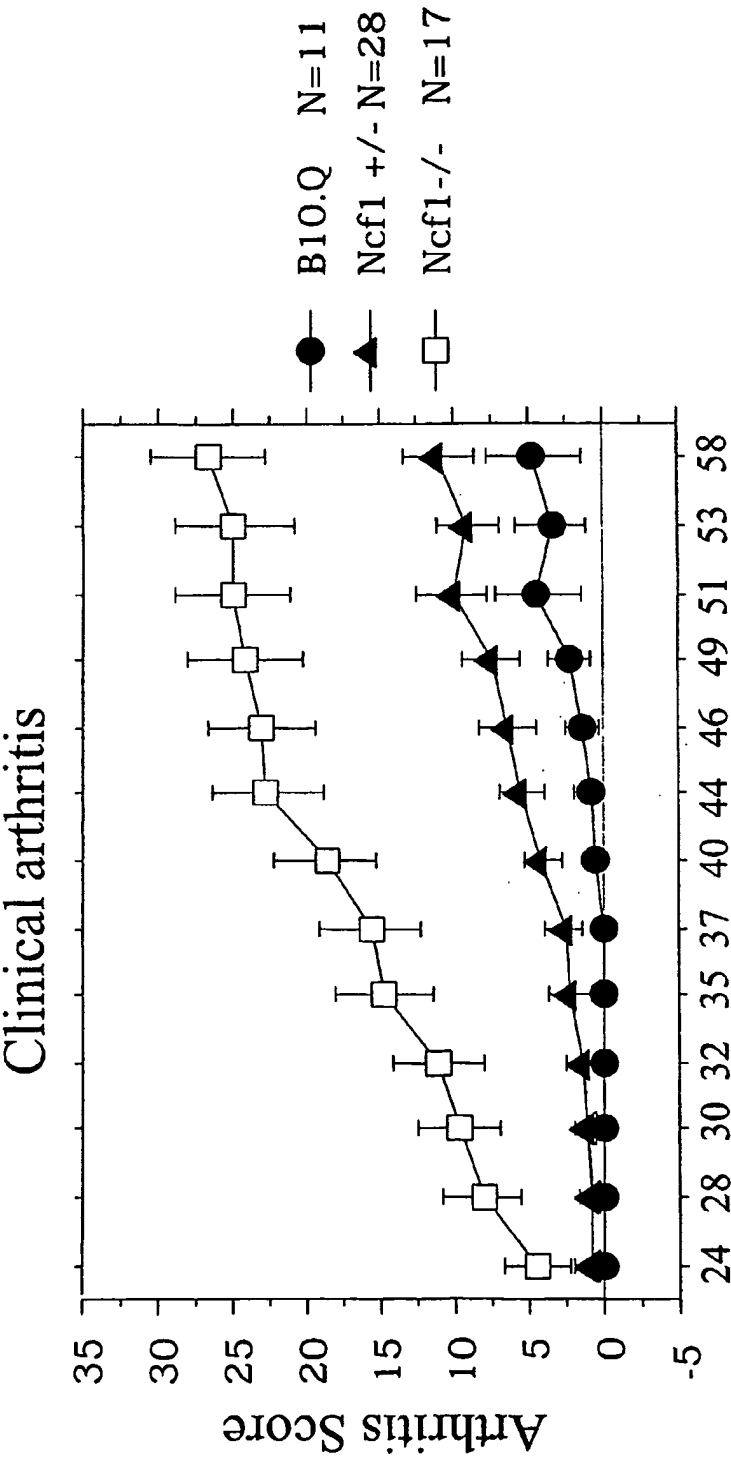
Figure 18A

Figure 18B

Maximal arthritis score
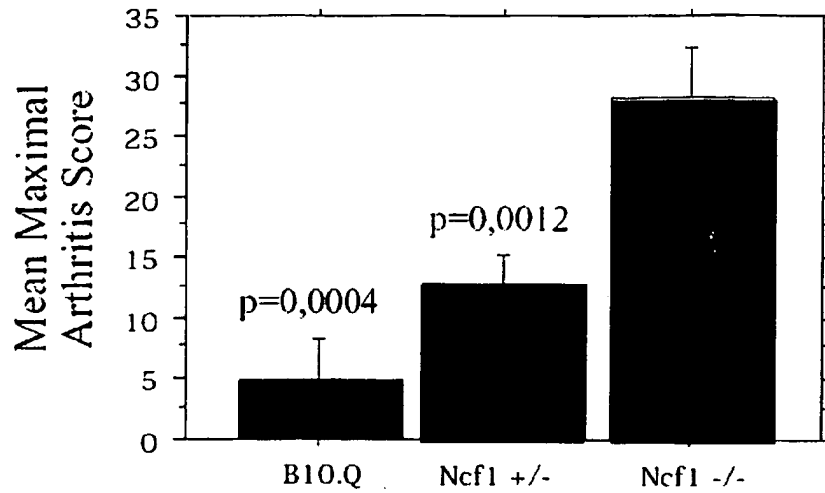
Figure 18C

Accumulated arthritis score
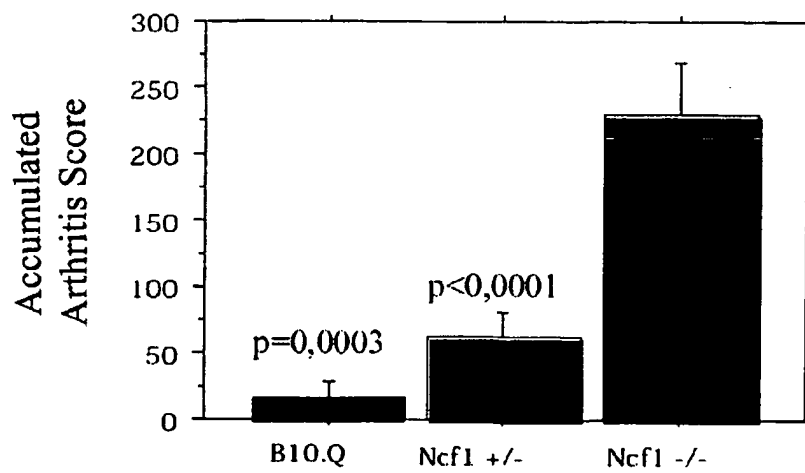
Figure 19

Onset of disease
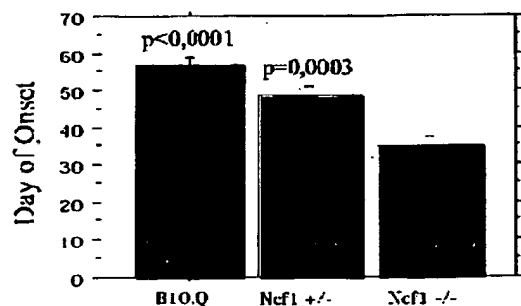
Figure 20

Serum COMP
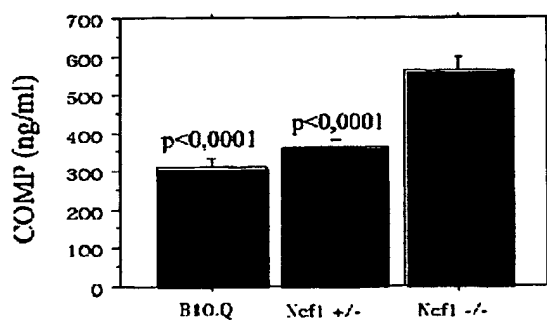
Figure 21

anti collagen antibodies
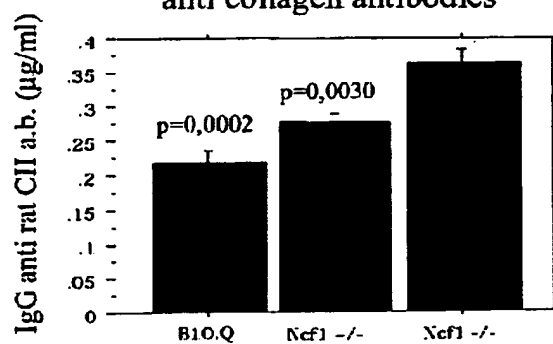
Figure 22

CIA in rats treated with phytol
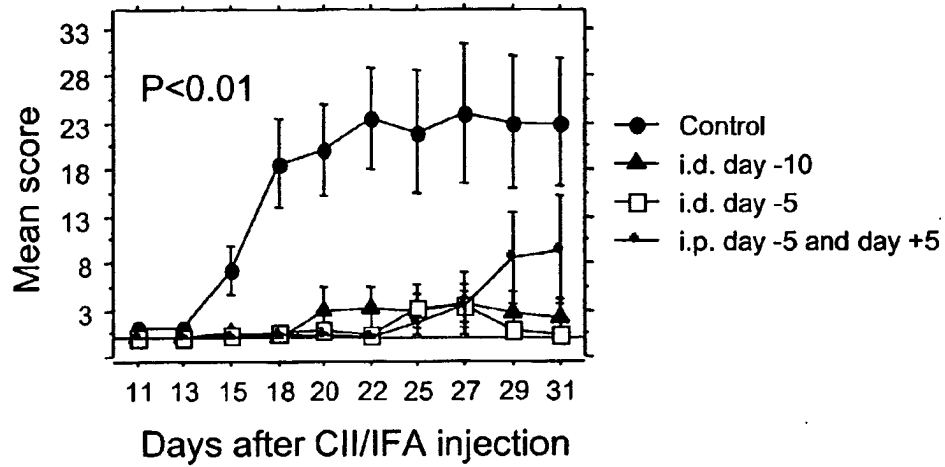
Figure 23A

Treatment of active PIA with phytol
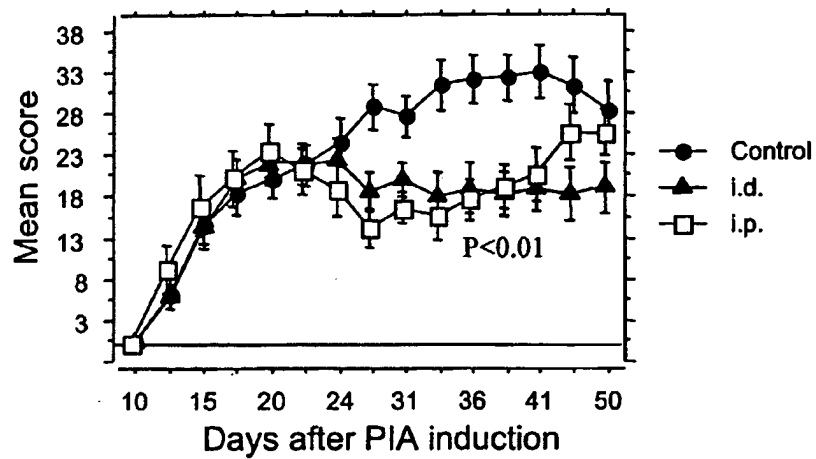
Figure 23B

Treatment of active PIA with undecane
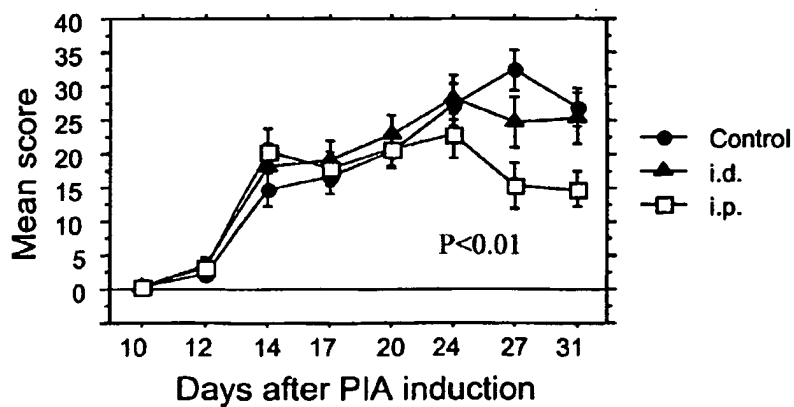
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agacctatcg ggccatcgct gactacgaga agggttccaa aacagagatg accgtggcga     540
cgggagatgt ggtggatgtc gtagagaaaa gcgagagtgg ctggtggttt tgccagatga     600
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tcctagatgg ctggtgggtg gtcaggaaag gggacatcac cggctacttc ccatccatgt     840
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caccacctcg caggtcgacc atccgcaatg cacagagcat ccaccagcgt tctcggaagc     960
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<210> 4
<211> 389
<212> PRT
<213> Rattus norvegicus

<400> 4
Met Gly Asp Thr Phe Ile Arg His Ile Ala Leu Leu Gly Phe Glu Lys
1               5                   10                  15
Arg Phe Val Pro Ser Gln His Tyr Val Tyr Met Phe Leu Val Lys Trp
                20                  25                  30
Gln Asp Leu Ser Glu Lys Val Val Tyr Arg Lys Phe Thr Glu Ile Tyr
            35                  40                  45
Glu Phe His Lys Met Leu Lys Glu Met Phe Pro Ile Glu Ala Gly Glu
        50                  55                  60
Ile His Thr Glu Asn Arg Val Ile Pro His Leu Pro Ala Pro Arg Trp
65                  70                  75                  80
Tyr Asp Gly Gln Arg Ala Ala Glu Ser Arg Gln Gly Thr Leu Thr Glu
                85                  90                  95
Tyr Phe Asn Ser Leu Met Gly Leu Pro Val Lys Ile Ser Arg Cys Pro
                100                 105                 110
His Leu Leu Asn Phe Phe Lys Val Arg Pro Asp Asp Leu Lys Leu Pro
        115                 120                 125
Asn Asp Ser Gln Val Lys Lys Pro Glu Thr Tyr Leu Thr Ala Lys Asp
    130                 135                 140
Gly Lys Asn Asn Val Ala Asp Ile Thr Gly Pro Ile Ile Leu Gln Thr
145                 150                 155                 160
Tyr Arg Ala Ile Ala Asp Tyr Glu Lys Gly Ser Lys Thr Glu Met Thr
                165                 170                 175
Val Ala Thr Gly Asp Val Val Asp Val Val Glu Lys Ser Glu Ser Gly
                180                 185                 190
Trp Trp Phe Cys Gln Met Lys Thr Lys Arg Gly Trp Val Pro Ala Ser
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          195                    200                    205
Tyr Leu Glu Pro Leu Asp Ser Pro Asp Glu Ala Glu Asp Pro Asp Pro
    210                    215                    220
Asn Tyr Ala Gly Glu Pro Tyr Val Thr Ile Lys Ala Tyr Ala Ala Val
225                    230                    235                    240
Glu Glu Asp Glu Val Ser Leu Ser Glu Gly Glu Ala Ile Glu Val Ile
                245                    250                    255
His Lys Leu Leu Asp Gly Trp Trp Val Val Arg Lys Gly Asp Ile Thr
                260                    265                    270
Gly Tyr Phe Pro Ser Met Tyr Leu Gln Lys Ala Gly Glu Glu Ile Thr
            275                    280                    285
Gln Ala Gln Arg Gln Ile Arg Ser Arg Gly Ala Pro Pro Arg Arg Ser
    290                    295                    300
Thr Ile Arg Asn Ala Gln Ser Ile His Gln Arg Ser Arg Lys Arg Leu
305                    310                    315                    320
Ser Gln Asp Thr Tyr Arg Arg Asn Ser Val Arg Phe Leu Gln Gln Arg
                325                    330                    335
Arg Arg Pro Ala Arg Pro Gly Pro Gln Ser Pro Asp Ser Lys Asp Asn
                340                    345                    350
Pro Ser Thr Pro Arg Ala Lys Pro Gln Pro Ala Val Pro Pro Arg Pro
            355                    360                    365
Ser Ser Asp Leu Ile Leu His Arg Cys Thr Glu Ser Thr Lys Arg Lys
    370                    375                    380
Leu Thr Ser Ala Val
385
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<210> 5
<211> 1349
<212> DNA
<213> Homo sapiens

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ctttgagaag cgcttcgtac ccagccagca ctatgtgtac atgttcctgg tgaaatggca   120
ggacctgtcg gagaaggtgg tctaccggcg cttcaccgag atctacgagt tccataaaac   180
cttaaaagaa atgttcccta ttgaggcagg ggcgatcaat ccagagaaca ggatcatccc   240
ccacctccca gctcccaagt ggtttgacgg gcagcgggcc gccgagaacc gccagggcac   300
acttaccgag tactgcagca cgctcatgag cctgcccacc aagatctccc gctgtcccca   360
cctcctcgac ttcttcaagg tgcgccctga tgacctcaag ctccccacgg acaaccagac   420
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catcctgaac cgctgcagcg agagcaccaa gcggaagctg gcgtctgccg tctgaggctg  1200
gagcgcagtc cccagctagc gtctcggccc ttgccgcccc gtgcctgtac atacgtgttc  1260
tatagagcct ggcgtctgga cgccgagggc agccccgacc cctgtccagc gcggctcccg  1320
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<210> 6
<211> 390
<212> PRT
<213> Homo sapiens

<400> 6
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                20                  25                  30
Gln Asp Leu Ser Glu Lys Val Val Tyr Arg Arg Phe Thr Glu Ile Tyr
            35                  40                  45
Glu Phe His Lys Thr Leu Lys Glu Met Phe Pro Ile Glu Ala Gly Ala
        50                  55                  60
Ile Asn Pro Glu Asn Arg Ile Ile Pro His Leu Pro Ala Pro Lys Trp
65                  70                  75                  80
Phe Asp Gly Gln Arg Ala Ala Glu Asn Arg Gln Gly Thr Leu Thr Glu
                85                  90                  95
Tyr Cys Ser Thr Leu Met Ser Leu Pro Thr Lys Ile Ser Arg Cys Pro
                100                 105                 110
His Leu Leu Asp Phe Phe Lys Val Arg Pro Asp Asp Leu Lys Leu Pro
            115                 120                 125
Thr Asp Asn Gln Thr Lys Lys Pro Glu Thr Tyr Leu Met Pro Lys Asp
        130                 135                 140
Gly Lys Ser Thr Ala Thr Asp Ile Thr Gly Pro Ile Ile Leu Gln Thr
145                 150                 155                 160
Tyr Arg Ala Ile Ala Asp Tyr Glu Lys Thr Ser Gly Ser Glu Met Ala
                165                 170                 175
Leu Ser Thr Gly Asp Val Val Glu Val Val Glu Lys Ser Glu Ser Gly
            180                 185                 190
Trp Trp Phe Cys Gln Met Lys Ala Lys Arg Gly Trp Ile Pro Ala Ser
            195                 200                 205
Phe Leu Glu Pro Leu Asp Ser Pro Asp Glu Thr Glu Asp Pro Glu Pro
        210                 215                 220
Asn Tyr Ala Gly Glu Pro Tyr Val Ala Ile Lys Ala Tyr Thr Ala Val
225                 230                 235                 240
Glu Gly Asp Glu Val Ser Leu Leu Glu Gly Glu Ala Val Glu Val Ile
                245                 250                 255
His Lys Leu Leu Asp Gly Trp Trp Val Ile Arg Lys Asp Asp Val Thr
            260                 265                 270
Gly Tyr Phe Pro Ser Met Tyr Leu Gln Lys Ser Gly Gln Asp Val Ser
            275                 280                 285
Gln Ala Gln Arg Gln Ile Lys Arg Gly Ala Pro Pro Arg Arg Ser Ser
        290                 295                 300
Ile Arg Asn Ala His Ser Ile His Gln Arg Ser Arg Lys Arg Leu Ser
305                 310                 315                 320
Gln Asp Ala Tyr Arg Arg Asn Ser Val Arg Phe Leu Gln Gln Arg Arg
                325                 330                 335
Arg Gln Ala Arg Pro Gly Pro Gln Ser Pro Gly Ser Pro Leu Glu Glu
            340                 345                 350
Glu Arg Gln Thr Gln Arg Ser Lys Pro Gln Pro Ala Val Pro Pro Arg
            355                 360                 365
Pro Ser Ala Asp Leu Ile Leu Asn Arg Cys Ser Glu Ser Thr Lys Arg
        370                 375                 380
Lys Leu Ala Ser Ala Val
385                 390
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<210> 7
<211> 390
<212> PRT
<213> Homo sapiens


<400> 7
Met Gly Asp Thr Phe Ile Arg His Ile Ala Leu Leu Gly Phe Glu Lys
1               5                   10                  15
Arg Phe Val Pro Ser Gln His Tyr Val Tyr Met Phe Leu Val Lys Trp
            20                  25                  30
Gln Asp Leu Ser Glu Lys Val Val Tyr Arg Arg Phe Thr Glu Ile Tyr
        35                  40                  45
Glu Phe His Lys Thr Leu Lys Glu Met Phe Pro Ile Glu Ala Gly Ala
    50                  55                  60
Ile Asn Pro Glu Asn Arg Ile Ile Pro His Leu Pro Ala Pro Lys Trp
65                  70                  75                  80
Phe Asp Gly Gln Arg Ala Ala Glu Asn Arg Gln Gly Thr Leu Thr Glu
                85                  90                  95
Tyr Cys Ser Thr Leu Met Ser Leu Pro Thr Lys Ile Ser Arg Cys Pro
            100                 105                 110
His Leu Leu Asp Phe Phe Lys Val Arg Pro Asp Asp Leu Lys Leu Pro
        115                 120                 125
Thr Asp Asn Gln Thr Lys Lys Pro Glu Thr Tyr Leu Met Pro Lys Asp
    130         .           135                 140
Gly Lys Ser Thr Ala Thr Asp Ile Thr Gly Pro Ile Ile Leu Gln Ser
145                 150                 155                 160
Tyr Arg Ala Ile Ala Asn Tyr Glu Lys Thr Ser Gly Ser Glu Met Ala
                165                 170                 175
Leu Ser Thr Gly Asp Val Val Glu Val Val Glu Lys Ser Glu Ser Gly
            180                 185                 190
Trp Trp Phe Cys Gln Met Lys Ala Lys Arg Gly Trp Ile Pro Ala Ser
            195                 200                 205
Phe Leu Glu Pro Leu Asp Ser Pro Asp Glu Thr Glu Asp Pro Glu Pro
    210     .           215                 220
Asn Tyr Ala Gly Glu Pro Tyr Val Ala Ile Lys Ala Tyr Thr Ala Val
225                 230                 235                 240
Glu Gly Asp Glu Val Ser Leu Leu Glu Gly Glu Ala Val Glu Val Ile
            245                 250                 255
His Lys Leu Leu Asp Gly Trp Trp Val Ile Arg Lys Asp Asp Val Thr
            260                 265                 270
Gly Tyr Phe Pro Ser Met Tyr Leu Gln Lys Ser Gly Gln Asp Val Ser
            275                 280                 285
Gln Ala Gln Arg Gln Ile Lys Arg Gly Ala Pro Pro Arg Arg Ser Ser
    290                 295                 300
Ile Arg Asn Val His Ser Ile His Gln Arg Ser Arg Lys Arg Leu Ser
305                 310                 315                 320
Gln Asp Ala Tyr Arg Arg Asn Ser Val Arg Phe Leu Gln Gln Arg Arg
                325                 330                 335
Arg Gln Ala Arg Pro Gly Pro Gln Ser Pro Gly Ser Pro Leu Glu Glu
            340                 345                 350
Glu Arg Gln Thr Gln Arg Ser Lys Pro Gln Pro Ala Val Pro Pro Arg
            355                 360                 365
Pro Ser Ala Asp Leu Ile Leu Asn Arg Cys Ser Glu Ser Thr Lys Arg
    370                 375                 380
Lys Leu Ala Ser Ala Val
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385   390

<210> 8
<211> 17302
<212> DNA
<213> Homo sapiens

<400> 8
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cttggctttc atctgccaga cctcaaccct cacccccagg agatcaggtc cggaccatga   180
gctgaccctg gactcaggca agggtgagtt ggtgcagccc tggcctgctg ggaggcacag   240
gctgcagcag gctgcctggg gctgaggccc gccactcatg aactcatgac cttgaatgag   300
ctccaaaagc tctgggcctc ccaggctcta gggggagtgg gagagagagg cctcagcctg   360
tccctgggca tgctgccccc tcctcacctc tttgtcccaa atccccttcc tggcaaagct   420
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cactcccatg cccgcgttca ttcactcatt cattcattca ttcactcatt cattgactca   660
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tgggaggcag aggcaggagg atcactagag gccagtagtt tgagaccatc ctgggcaaca   1200
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